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Article

Performance Evaluation of Instrument-Based SARS-CoV-2 Rapid Antigen Fluorescent Immunoassays for Point-of-Care Detection

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Abstract

Rapid antigen tests targeting SARS-CoV-2 nucleocapsid protein were essential for decentralised testing during the COVID-19 pandemic. Independent performance evaluations are critical to guide regulatory approval and implementation, particularly in resource-limited settings. This study presents a retrospective analytical and operational evaluation of two instrument-based fluorescent immunoassays (FIA): the PCL COVID-19 Ag Rapid FIA (PCL Inc., South Korea) and LumiraDx SARS-CoV-2 Ag Test (LumiraDx Ltd., UK). Analytical sensitivity was determined using recombinant nucleocapsid protein and viral cultures. Clinical performance was assessed using residual clinical specimens (n=110) with RT-PCR as reference, stratified by cycle threshold (Ct). Operational characteristics were assessed using a structured Likert framework. Overall sensitivity was 63% (51-73) for PCL and 95% (88-99) for LumiraDx. For Ct≤25, sensitivity increased to 93% and 100%. Specificity was ≥97% for both. LumiraDx maintained sensitivity (83-94%) at Ct 25-30, whereas PCL detected no positives in this range. Limit of detection was 0.039 nM (PCL) and 0.6 pM (LumiraDx). Operational usability was high for both (90% PCL, 87% LumiraDx). LumiraDx showed higher analytical sensitivity across a broader viral load range, supporting primary diagnostic use, whereas PCL was limited to high viral loads. This provides a reproducible framework for rapid diagnostic assessment during emerging outbreaks.

Keywords: SARS-CoV-2; COVID-19; rapid antigen test; fluorescent immunoassay; point-of-care testing; diagnostic accuracy; pandemic preparedness

1. Introduction

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in 2019 placed unprecedented strain on global diagnostic systems, often exceeding the capacity of centralised molecular testing platforms. Although more than 779 million cases have been confirmed through laboratory testing worldwide [1], seroprevalence studies indicate that the true level of population exposure is much higher [2,3]. This discrepancy highlights limitations in testing accessibility and capacity, especially during the early phases of the pandemic. Low- and middle-income countries experienced significant challenges in scaling up reverse transcription polymerase chain reaction (RT-PCR) testing, including limited laboratory infrastructure, supply chain disruptions, and global shortages of critical [4,5]. These constraints emphasised the need for alternative diagnostic approaches that could be rapidly deployed outside of centralised laboratory settings.

Rapid antigen tests targeting the conserved nucleocapsid (N) protein emerged as a key solution for decentralised testing. These assays enabled rapid clinical decision-making, patient triage, and large-scale community screening [6,7]. The global diagnostics landscape expanded rapidly, with over 1,000 antigen-based assays developed across multiple platforms [8]. In response, regulatory bodies

such as the U.S. Food and Drug Administration (FDA), the European Commission, and the World Health Organization (WHO) established emergency use pathways and performance guidelines [9–11] to facilitate rapid deployment [12]. Minimum performance criteria typically included $\geq 80\%$ sensitivity and $\geq 97\%$ specificity [13], alongside operational requirements such as ease of use, short turnaround time (≤ 30 minutes), and suitability for point-of-care implementation.

The South African Health Products Regulatory Authority (SAHPRA) received numerous SARS-CoV-2 antigen rapid test applications under emergency use conditions. Among these were assays based on fluorescent immunoassay (FIA) technology, which requires instrument-based result interpretation. While conventional lateral flow immunochromatographic assays offer advantages in cost and simplicity, they are limited by subjective visual interpretation and manual data capture, increasing the risk of user-dependent variability [14,15]. In contrast, instrument-based FIAs provide automated signal detection, standardised result interpretation, and integrated data management capabilities, supporting improved accuracy and digital health integration [16].

Despite manufacturer-reported performance data, independent evaluations remain essential to verify diagnostic accuracy under local conditions and to inform regulatory decision-making. Variability in assay performance across different settings, specimen types, and viral load distributions necessitate context-specific validation.

In this study, we present a retrospective performance evaluation of two instrument-based FIAs: the PCL COVID19 Ag Rapid FIA (PCL Inc., South Korea) and the LumiraDx SARS-CoV-2 Ag Test (LumiraDx Ltd., UK). We evaluated analytical sensitivity using recombinant protein and viral culture and evaluated clinical performance using residual patient specimens stratified by RT-PCR Ct values. In addition, operational characteristics were assessed using a structured framework to determine suitability for point-of-care deployment. Key characteristics of the evaluated assays are summarised in Table 1.

Table 1. Key features of the SARS-CoV-2 antigen assays evaluated in this study.

Characteristics	PCL COVID19 Ag Rapid FIA	LumiraDx SARS-CoV-2 Ag Test
Regulatory certification (at time of evaluation)	SAHPRA (08/01/2021), TGA (30/07/2021)	FDA EUA (18/12/2020)
Target population	Symptomatic individuals	Symptomatic and asymptomatic individuals
Specimen type(s)	Nasopharyngeal/Oropharyngeal	Nasal/Nasopharyngeal
Format/design	Immunosandwich lateral flow assay	Immunosandwich microfluidic assay
Target SARS-CoV-2 protein	Undisclosed	Nucleocapsid protein
Sample volume applied to test cassette	4 drops	1 drop (~20 μ L)
Result interpretation	PCLOK EZ instrument	LumiraDx instrument
Time to result	Standard mode: 10 min on-board incubation; Quick mode: 10 min bench incubation + instrument read	12 minutes after test strip insertion
Additional features	On-board printer; barcode scanning; USB data export	Automated quality checks; RFID calibration; cloud-based connectivity; LIS integration
Manufacturer performance data*	89% sensitivity and 99% specificity	98% sensitivity and 97% specificity

*Obtained using fresh clinical specimens in accordance with the IFU. FIA – Fluorescent immunoassay, SAHPRA – South African Health Products Regulatory Authority, TGA – Therapeutic Goods Administration, EUA –

Emergency Use Authorisation, FDA – U.S. Food and Drug Administration, WHO EUL – World Health Organisation Emergency Use Listing, RFID – Radio Frequency Identification, LIS – Laboratory Information System.

2. Materials and Methods

2.1. SARS-CoV-2 Purified Recombinant Protein Panel

Purified recombinant SARS-CoV-2 wild-type N protein (~60.68 kDa) was provided by Prof. Jonathan Blackburn (University of Cape Town, South Africa). The protein was expressed in a baculovirus expression system using KREX™ functional proteomics technology (Sengenics Co Pte Ltd., UK). To determine the limit of detection (LoD) for each assay, N protein concentrations ranging from 10nM to 2.4pM were prepared in manufacturer specific assay buffers. Each concentration was tested in duplicate according to the respective manufacturer’s instructions.

2.2. SARS-CoV-2 Viral Culture Panel

SARS-CoV-2 wild-type viral culture (2019-nCoV strain) was obtained through collaborators in South Africa (Prof. Wolfgang Preiser, Stellenbosch University; Prof. Bavesh Kana, University of the Witwatersrand). Viral stock was semi-quantified using reference RNA material (~log10 5 viral copies/μL). Two dilutions (1×10³ and 1×10⁴) were prepared, corresponding to approximately log 5.9 viral copies/mL and log10 4.7 viral copies/mL, respectively, following previously described methods [17]. Each dilution was mixed 1:1 with the respective assay buffer and tested according to manufacturer instructions. Parallel RT-PCR testing was performed using the TaqPath™ COVID-19 CE-IVD RT-PCR Kit on the QuantStudio platform (ThermoFisher Scientific, Waltham, MA, USA) to obtain corresponding Ct values.

2.3. Residual Clinical Specimen Evaluation Panels

Residual nasopharyngeal clinical specimens were obtained from routine SARS-CoV-2 RT-PCR testing at the HIV Molecular Laboratory of the National Health Laboratory Service, Charlotte Maxeke Johannesburg Academic Hospital, Johannesburg, South Africa. Ethics approval for use of residual specimens was granted by the University of the Witwatersrand Human Research Ethics Committee (approval #M1911201). Swabs were received at the testing laboratory and processed in phosphate-buffered saline (PBS), saline, viral transport medium (VTM), or universal transport medium (UTM), and tested using the cobas® SARS-CoV-2 platform (Roche Molecular, Pleasanton, CA, USA). Specimens were stratified based on Ct values into high viral load (HVL; Ct ≤25), medium viral load (MVL; Ct 25-30), low viral load (LVL; Ct 30-35), and SARS-CoV-2 negatives. A total of 110 specimens were evaluated per assay panel. Panel composition and specimen distribution are summarised in Table 2.

Table 2. Description of the SARS-CoV-2 challenge panel materials.

Material		Description	
SARS-CoV-2	purified	10 nM, 2.5 nM, 0.625 nM, 0.039 nM, 9.75 pM and 2.4 pM concentrations	
	recombinant N protein	tested in duplicate	
SARS-CoV-2	viral	1x10 ³ and 1x10 ⁴ dilutions tested in triplicate and compared to RT-PCR cultures	
SARS-CoV-2	residual clinical specimens	PCL COVID19 Ag Rapid FIA	LumiraDx SARS-CoV-2 Ag Test
		Collected: Oct - Dec 2020	Collected: April - June 2021

	N=110	N=110
	n=54 HVL, n=10 MVL, n=17 LVL, n=30 Neg	n=42 HVL, n=18 MVL, n=20 LVL, n=30 neg
	Panel comprised of SA wave 1* specimens	Panel comprised of SA wave 1 and wave 2* specimens
Methods applied	Simulated swab into kit buffer (A); 1:1 dilution of specimen with kit buffer (B)	Simulated swab into kit buffer (A); 1:7 dilution of specimen with kit buffer (C)

*South African wave 1 was associated with a mix of SARS-CoV-2 lineages, whereas wave 2 comprised predominantly beta strains (B.1.351). .

Due to the use of residual specimens, modifications to manufacturer protocols were required. Three specimen preparation methods were evaluated. Method A: Simulated swab protocol using a sterile swab (provided with test kits) were immersed in residual specimen for approximately 1 minute before processing according to manufacturer instructions. Method B: Direct 1:1 dilution of residual specimen in assay buffer prior to testing. Method C: Serial dilution of specimens (1:2-1:9) in assay buffer; for LumiraDx, a 1:7 dilution was selected based on manufacturer guidance and used for final analysis.

Although the assays were evaluated using different specimen panels, comparability was supported by standardisation of Ct distributions and validation against comparator assays. Discordant results were excluded following consensus review.

2.4. Precision Analysis

Intra-assay precision was assessed using quintuplicate testing of four residual clinical specimens (two SARS-CoV-2 positive with Ct <30 and two negative). Each specimen was tested five times within a single day (total n=20 per assay).

2.5. Statistical Analysis

Diagnostic performance, including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and Cohen’s kappa coefficient (κ), were calculated using STATA version 14 (StataCorp, USA). Agreement was interpreted using the Landis and Koch scale: poor (<0.2), fair (0.2–0.4), moderate (0.4–0.6), good (0.6–0.8), and very good (0.8–1.0). Confidence intervals (95% CI) were calculated for all relevant estimates.

2.6. Operational Assessment

Operational characteristics were evaluated using a structured 5-point Likert scale (1=very poor/difficult; 5 = very good/easy), across the following domains (i) kit contents ready for implementation (including protective materials and consumables); (ii) biosafety requirements (safe use without specialised facilities); (iii) training complexity (standard procedure, no specialised skills); (iv) ease of use from specimen collection to result interpretation; (v) time to result (acceptable $\leq$ 40 min, optimal $\leq$ 20 min); and (vi) invalid/error rate (acceptable $\leq$ 5%). Scoring was performed by laboratory operators following completion of assay evaluations.

3. Results

3.1. Performance Using Reference Materials

The analytical performance of the PCL and LumiraDx SARS-CoV-2 antigen assays were first evaluated using viral culture and purified recombinant nucleocapsid protein. Both assays successfully detected SARS-CoV-2 viral culture at a dilution of 1×10^3 (~log10 5.9 viral copies/mL),

corresponding to RT-PCR Ct values ≤ 25 . Neither assay detected the lower concentration dilution (1×10^4 ; $\sim \log_{10}$ 4.8 viral copies/mL), which correspond to Ct values ranging from 25–28 (Table 3a).

Using purified recombinant N protein, the LoD differed substantially between assays. The PCL assay demonstrated a LoD of 0.039 nM (0.36 ng/ μ L), whereas the LumiraDx assay detected concentrations as low as 0.6 pM (0.006 ng/ μ L), indicating a higher analytical sensitivity (Table 3b).

Table 3. Analytical performance of SARS-CoV-2 antigen assays using (a) viral cultures and (b) purified recombinant N protein.

(a) SARS-CoV-2 viral cultures (SA wild-type)								
Assay		Dilution		FIA	N	S	ORF1ab	
		factor		result				
PCL COVID19 Ag Rapid FIA		1x10 ³		Positive	24,1	24,6		23,9
		1x10 ⁴		Negative	27,2	27,9		27,1
LumiraDx SARS-CoV-2 Ag Test		1x10 ³		Positive	24,59	24,09		23,90
		1x10 ⁴		Negative	27,86	27,23		27,13
(b) SARS-CoV-2 purified recombinant nucleocapsid proteins (SA wild-type)								
Protein Concentration	10 nM	2.5 nM	0.625 nM	0.039 nM	9.75 pM	2.4 pM	0.6 pM	0.15 pM
PCL COVID19 Ag Rapid FIA	Positive	Positive	Positive	Positive	Negative	Negative	Negative	Negative
LumiraDx SARS-CoV-2 Ag Test	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Negative

3.2. Performance Using Residual Clinical Specimens

A total of 110 residual clinical specimens were evaluated for each assay. Compared to RT-PCR, the overall diagnostic sensitivity (95% CI) was 63% (51-73) for the PCL assay and 95% (88-99) for the LumiraDx assay (Table 4). Specificity was 100% (88-100) for PCL and 97% (83-100) for LumiraDx.

Stratification by viral load demonstrated a strong dependence of assay sensitivity on Ct values. For HVL specimens ($Ct \leq 25$), sensitivity increased to 93% (82-98) for PCL (Method B) and reached 100% (92-100) for LumiraDx across both evaluated methods. In the MVL range (Ct 25-30), performance varied substantially between assays. LumiraDx maintained high sensitivity, achieving 83% using Method A and 94% using Method C. Whereas, PCL assay did not detect any positive specimens within this Ct range. For LVL specimens (Ct 30–35), LumiraDx retained moderate detection capability (65-85%, depending on method), whereas PCL performance remained limited. The distribution of antigen test results in relation to Ct values is presented in Figure 1.

Table 4. Analytical performance of SARS-CoV-2 antigen assays on residual clinical specimens.

Assay	Method	Ct range	n	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Cohen Kappa (95% CI)	Agreement score
PCL COVID19 Ag Rapid	A and B	Overall performance	110	63% (51-73)	100% (88-100)	100% (93-100)	50% (37-63)	0.48 (0.34-0.61)	Moderate
	A	$Ct \leq 25$	53 pos, 30 neg	91% (79-97)	100% (88-100)	100% (93-100)	86% (70-95)	0.87 (0.77-0.98)	Very Good

d			53	93% (82-98)	100% (88-100)	100% (93-100)	88% (73-97)	0.89 (0.80-1.00)	Very Good
FIA	B	Ct ≤ 25	pos, 30 neg						
	A, B	Ct 25-30	7 pos, 30 neg	No positives detected (0%)/ All negatives correctly identified (100%)					
	A, B	Ct 30-35	20 pos, 30 neg						
	A and C	Overall performance	110	95% (88-99)	97% (83-100)	99% (93-100)	88% (72-97)	0.89 (0.79-0.98)	Very Good
	A	Ct ≤ 25	42 pos, 30 neg	100% (92-100)	97% (83-100)	98% (88-100)	100% (88-100)	0.97 (0.92-1.02)	Very Good
LumiraD x SAR S-CoV-2 Ag Test	A	Ct 25-30	18 pos, 30 neg	83% (59-96)	97% (83-100)	94% (70-100)	91% (75-98)	0.97 (0.92-1.03)	Very Good
	A	Ct 30-35	20 pos, 30 neg	65% (41-85)	97% (83-100)	93% (66-100)	81% (64-92)	0.82 (0.65-0.99)	Very Good
	C	Ct ≤ 25	42 pos, 30 neg	100% (92-100)	97% (83-100)	98% (88-100)	100% (88-100)	0.91 (0.80-1.03)	Very Good
	C	Ct 25-30	18 pos, 30 neg	94% (73-100)	97% (83-100)	94% (73-100)	97% (83-100)	0.65 (0.43-0.87)	Good
	C	Ct 30-35	20 pos, 30 neg	85% (62-97)	97% (83-100)	94% (73-100)	91% (75-98)	0.83 (0.67-0.99)	Very Good

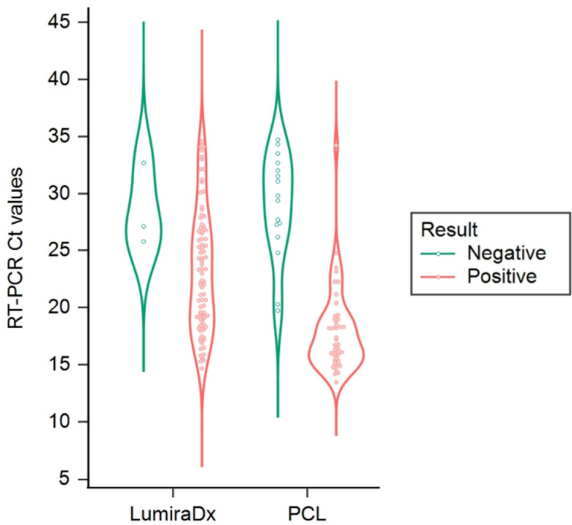


Figure 1. Distribution of positive and negative rapid antigen test results by RT-PCR Ct value (Violin plot with jittered data points showing the distribution of positive (red) and negative (green) rapid antigen test results for both assays in relation to corresponding RT-PCR Ct values (y-axis)).

3.3. Precision

Intra-assay precision was high for both assays. All positive and negative specimens (100%) were correctly classified across quintuplicate testing, demonstrating excellent repeatability. Additionally, LumiraDx quality controls and reference panel specimens (MRNDx) produced results consistent with manufacturer specifications.

3.4. Operational Performance

Operational performance, assessed using a structured Likert scale, yielded high overall scores for both assays: 90% (27/30) for PCL and 87% (26/30) for LumiraDx (Table 5). Both assays scored highly for ease of use, minimal training requirements, and rapid time to result. However, workflow limitations were noted. Instrument-based processing in single-read mode increased total turnaround time for both platforms. The LumiraDx system occasionally generated “insufficient sample volume” errors despite adequate sample application, contributing to a slightly lower score for invalid/error rate.

Table 5. Operational performance of SARS-CoV-2 Ag assays.

Characteristic	PCL COVID19 Ag Rapid FIA	LumiraDx SARS-CoV-2 Ag Test
Kit contents ready for implementation	4	4
Need for biosafety	4	4
Training required	5	5
Ease of use from specimen collection to result interpretation	4*	4‡
Time to result	5	5
Invalid (error rate)	5	4*
Comments	* Testing time increased with single-read mode	‡Testing time increased with single-read cartridge *Analyser displayed “sample error - insufficient volume” despite adequate well coverage

4. Discussion

This study presents a structured, independent evaluation of two instrument-based SARS-CoV-2 antigen tests conducted during a public health emergency. Using a standardised framework that includes analytical, clinical, and operational components, we demonstrate that while both assays meet minimum performance thresholds for HVL detection, their diagnostic utility differs substantially across the viral load ranges.

Both assays achieved high sensitivity in specimens with Ct ≤25, which corresponds to peak viral load and the period of high infectiousness. This finding aligns with established data reporting that antigen test sensitivity is highest during early infection when viral replication is at peak [18]. Reliable detection within this range supports the use of both assays for identifying individuals most likely to transmit infection [19,20], reinforcing their role in rapid clinical triage and outbreak response.

However, a key difference between the assays occurred at lower viral loads. The LumiraDx assay maintained high sensitivity (83-94%) in the MVL range (Ct 25-30), whereas the PCL assay failed to detect specimens within this category. This difference is clinically important, as individuals within this Ct range may still contribute to transmission, especially during the early or recovery phase [21,22]. The ability to detect these cases enhances the utility of antigen testing beyond detecting only highly infectious individuals.

The observed differences in clinical performance align with the analytical sensitivity results from this study. The approximately two-log difference in LoD between assays (0.6 pM for LumiraDx vs 0.039 nM for PCL) likely explains the improved performance of the LumiraDx platform at lower antigen concentrations. This increased sensitivity can be attributed to differences in assay design. The LumiraDx system employs a microfluidic platform with controlled sample processing and enhanced signal detection, whereas the PCL assay utilises a lateral flow format, which is more prone to matrix effects [23] and reduced sensitivity at low antigen levels.

These findings are consistent with previous studies evaluating SARS-CoV-2 antigen tests. Prior evaluations of lateral flow assays have reported sensitivities ranging from approximately 50-90%, with performance highly dependent on viral load, and the highest sensitivity observed at Ct $\leq$ 25 [24,25]. In contrast, instrument-based assays, including LumiraDx, have consistently demonstrated higher sensitivity and better correlation with viral culture positivity [26]. Meta-analyses have also identified LumiraDx as among the highest-performing antigen platforms [27], supporting the advantages of signal amplification and standardised detection.

The relationship between Ct value and antigen test positivity observed in this study further supports the use of Ct as a proxy for infectiousness [18]. The consistent detection of specimens with Ct $\leq$ 25 by both assays, along with the absence of detection at higher Ct values, reflects the biological relationship between viral load and antigen availability.

Beyond diagnostic accuracy, both assays demonstrated strong operational performance, confirming their suitability for decentralised testing. High scores for ease of use, minimal training needs, and quick turnaround times align with key criteria for point-of-care deployment. However, workflow limitations were noted. Both platforms operate in a single-read mode, which may limit throughput during high-demand periods. Additionally, the occurrence of “insufficient sample volume” errors with the LumiraDx system highlights the importance of operator training and system optimisation in real-world use.

An advantage of the LumiraDx platform is its ability to perform multiple tests, enabling the combination of infectious and non-communicable disease testing on a single device. This broadens its usefulness beyond just COVID-19 and encourages long-term integration into diagnostic networks, especially in resource-limited environments where platform flexibility is essential.

This study has several limitations. The lack of clinical metadata prevented analysis based on symptom duration or disease severity, which are known to impact viral dynamics and test performance. The use of multiple transport media may have influenced antigen stability and assay sensitivity. The assays were evaluated with different specimen panels, limiting direct comparison, though stratification by Ct values mitigated this constraint. Variant-specific performance was not systematically examined; however, using N protein targets decreases the chance of performance variation across variants. The use of residual clinical specimens required protocol adjustments, which might not fully reflect performance under manufacturer-recommended conditions. Lastly, limited sample sizes within stratified analyses could affect the precision of subgroup estimates.

Despite these limitations, this study offers a strong and reproducible framework for rapid diagnostic evaluation. The combined use of recombinant protein, viral culture, and clinical specimens allowed for a thorough assessment of both analytical and clinical performance. Stratification by Ct value provided clinically relevant insights into how the assay performs at different stages of infection, while a structured operational assessment highlighted practical considerations for implementation.

Importantly, these findings highlight the need for independent, context-specific evaluation within national regulatory processes. The variability in performance between assays with similar intended uses highlights the limitations of relying solely on manufacturer-reported data. The framework used in this study is scalable and adaptable, supporting quick assessment of diagnostic technologies during future infectious disease outbreaks.

5. Conclusions

Both evaluated SARS-CoV-2 antigen assays met the minimum performance criteria for detecting HVL infections, supporting their utility in identifying individuals at peak infectiousness. However, the LumiraDx assay showed higher sensitivity across a broader viral load range, including clinically relevant MVL specimens, supporting its use in near-patient diagnostic settings. Conversely, the PCL assay showed limited sensitivity beyond HVL specimens, indicating its suitability may be restricted to targeted screening strategies focused on highly infectious individuals. Instrument-based antigen assays offer important advantages over traditional lateral flow tests, including standardised result interpretation, reduced operator variability, and integration with digital health systems. Additionally, the multi-analyte capability of the LumiraDx platform enhances its long-term value by enabling broader diagnostic applications beyond COVID-19. The evaluation framework described in this study provides a practical and reproducible method for rapid diagnostic validation during public health emergencies. Incorporation of such standardised evaluation strategies into regulatory pathways will support the timely implementation of fit-for-purpose diagnostic technologies in resource-limited settings.

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Institutional Review Board Statement: Ethical approval was granted by the University of the Witwatersrand Human Research Ethics Committee (approval #M1911201).

Informed Consent Statement: Informed consent was not required as this study used residual de-identified specimens, and the use of these specimens for research was approved by the Institutional Review Board (approval #M1911201).

Data Availability Statement: All relevant data are presented within the manuscript. No publicly archived datasets were generated or analysed during this study.

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

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