

A Comparative Study of the Serological and Molecular Aspects of Toxoplasmosis in People Living with HIV in Kinshasa

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ABSTRACT

Introduction

Toxoplasmosis is a major public health concern in sub-Saharan Africa, particularly among people living with HIV (PLHIV), owing to the severe neurological complications and high mortality associated with the disease. Diagnosis primarily relies on detecting anti-*Toxoplasma gondii* antibodies or amplifying parasitic DNA via polymerase chain reaction (PCR).

Purpose

To evaluate and compare the diagnostic performance—including sensitivity, specificity, predictive values, diagnostic accuracy, and inter-test agreement—of serological and molecular methods for detecting toxoplasmosis among PLHIV in Kinshasa.

Methods

A descriptive cross-sectional study with an analytical component was conducted from October 2025 to January 2026 across public referral health centers in Kinshasa. A total of 71 PLHIV presenting with clinical signs suggestive of toxoplasmosis were enrolled. Anti-*T. gondii* IgG antibodies were detected using the Enzyme-Linked Fluorescent Assay (ELFA) on the Mini VIDAS system. Conventional PCR targeting the 529-bp repetitive element of *T. gondii* was performed on leukocyte DNA extracted using the PureLink Viral RNA/DNA Mini Kit and served as the reference method.

Results

IgG serology was positive in 32.4% (n=23) of participants, whereas PCR detected *T. gondii* DNA in 39.4% (n=28). Cross-tabulation revealed 19 true positives, 39 true negatives, 4 false positives, and 9 false negatives (seronegative/PCR-positive discordance: 12.7%). Diagnostic methods showed a statistically significant association ($\chi^2=23.94, p<.001$; Fisher's exact test $p<.001$; OR=19.38, 95% CI [4.93, 98.49]). IgG serology demonstrated a sensitivity of 67.9% (95% CI [49.3%, 82.1%]), specificity of 90.7% (95% CI [77.9%, 96.2%]), positive predictive value of 82.6% (95% CI [63.2%, 96.2%]), and negative predictive value of 81.3% (95% CI [68.1%, 89.4%]). The overall diagnostic accuracy was 81.7% (95% CI [70.7%, 89.8%]), with a Cohen's kappa coefficient of 0.60 (95% CI [0.39, 0.82]), indicating moderate agreement. Females constituted 59.2% (n=42) of the sample (female-to-male ratio of 1.4:1). The mean age was 44±12 years (range: 18–75 years), with the 36–45 age group being the most represented (28.2%).

Conclusion

Compared to IgG serology, PCR demonstrated superior diagnostic sensitivity for detecting active toxoplasmosis in immunocompromised patients. While PCR serves as a critical reference tool, serological and molecular modalities provide complementary clinical insights and should be integrated into combined diagnostic algorithms for PLHIV.

INTRODUCTION

Toxoplasmosis is among the most frequent opportunistic infections affecting people living with HIV (PLHIV), frequently leading to focal brain lesions, encephalopathy, coma, and mortality (Fang et al., 2021; Konaté et al., 2022). Globally, approximately one-third of the human population is chronically infected with *Toxoplasma gondii*, establishing toxoplasmosis as a ubiquitous zoonotic infection (Fera et al., 2020). The seroprevalence of anti-*T. gondii* antibodies exhibits broad geographic variation, ranging from 11% in North America to over 80% in regions of Latin America, Europe, and sub-Saharan Africa (National Institutes of Health [NIH], 2025). This variance stems from differences in climate, culinary habits, sanitation, and livestock management practices (Gelaw et al., 2024). Environmental dynamics and climate variability further influence transmission by altering the persistence and dissemination of infectious oocysts in soil and water systems (Zang et al., 2022).

In immunocompromised hosts, severe clinical disease typically results from the reactivation of encysted bradyzoites into replicating tachyzoites when cellular immunity drops significantly. Prior to widespread combination antiretroviral therapy (ART), the annual incidence of toxoplasmic encephalitis approached 33% among seropositive patients with severe CD4 T-cell depletion who were not receiving antimicrobial prophylaxis (NIH, 2025). Although uncommon in seronegative individuals, disease occurrence in this subset can stem from primary infection, impaired antibody synthesis due to advanced immunosuppression, or serological detection limits (NIH, 2025).

Sub-Saharan Africa bears approximately 87% of the global burden of HIV-*T. gondii* coinfection (World Health Organization [WHO], 2024). Despite this high burden, regional epidemiological data remain heterogeneous due to variable diagnostic protocols and restricted access to molecular testing. A meta-analysis across Africa highlighted high pooled seroprevalence rates, peaking in Central Africa (54.0%) and East Africa, with primary transmission linked to cat contact, raw vegetable consumption, and undercooked meat ingestion (Gelaw et al., 2024). In the Democratic Republic of the Congo (DRC), documented seroprevalence ranges between 40% and 80%

depending on the population sub-group and analytical assay utilized (Furaha et al., 2023; Mbo Duka et al., 2026).

Screening via anti-*T. gondii* IgG and IgM antibody assays is recommended during initial HIV intake to establish baseline exposure and guide prophylactic management. However, while automated serological methods are efficient and widely available, they present limitations: IgG antibodies persist indefinitely following initial exposure and cannot differentiate past immunity from active disease, whereas IgM antibody production is frequently blunted or absent during profound immunosuppression (Bongonya et al., 2020; NIH, 2025). Consequently, seronegativity cannot definitively exclude active opportunistic infection in severely immunocompromised individuals (NIH, 2025).

Direct amplification of *T. gondii* DNA via polymerase chain reaction (PCR) provides an alternative diagnostic path independent of host humoral responsiveness (NIH, 2025). PCR sensitivity varies by anatomical sampling site, target gene selection, parasite load, and timing of disease presentation (NIH, 2025). Nevertheless, its diagnostic specificity makes it valuable for identifying active infection when combined with clinical and radiological parameters. In resource-constrained settings such as Kinshasa, routine clinical implementation of PCR is restricted by instrument costs, reagent availability, and specialized laboratory infrastructure demands.

To address diagnostic gaps in local clinical settings, this study evaluated and compared the performance parameters—sensitivity, specificity, predictive values, accuracy, and agreement—of automated IgG serology against conventional molecular amplification in a cohort of PLHIV presenting with suspected clinical toxoplasmosis in Kinshasa.

METHODS

Study Setting, Design, and Period

This descriptive cross-sectional study with an analytical comparative component was conducted across public referral hospitals in Kinshasa, DRC, including the Ngaba Mother and Child Center, Elonga Health Center, and Vijana Health Center. Patient enrollment and laboratory

analyses took place over a 4-month period from October 2025 to January 2026.

Study Population and Eligibility Criteria

The study population comprised HIV-infected adults (≥ 18 years of age) presenting with clinical features suggestive of active toxoplasmosis. Clinical criteria included signs of encephalopathy (altered mental status, confusion, fever, spatial-temporal disorientation), signs of intracranial hypertension (headache, projectile vomiting, papilledema, seizures), or focal neurological deficits (hemiparesis, facial palsy, aphasia).

- i. **Inclusion criteria:** Confirmed HIV-1/2 positivity according to national diagnostic algorithms, clinical suspicion of active toxoplasmosis, age ≥ 18 years, and written informed consent.
- ii. **Exclusion criteria:** Patients under 18 years of age, those lacking clinical signs suggestive of active toxoplasmosis, or those unable/unwilling to provide informed consent.

Sampling and Specimen Processing

A non-probability convenience sample of 71 eligible patients was consecutively enrolled during the study period. From each participant, 5 mL of whole blood was collected via venipuncture into EDTA tubes and serum separator tubes. Samples were transported at 4°C to the Molecular Biology Unit at the University of Kinshasa. Serum was separated via centrifugation at 3,000 rpm for 5 minutes and allocated for serological testing. Leukocyte fractions (buffy coat) were processed onto filter paper to prepare Dried Buffy Coat Spots (DBCS) for nucleic acid extraction. Specimen aliquots were coded and stored at -20° C until testing.

Serological Testing

Anti-*T. gondii* IgG antibodies were measured using the Enzyme-Linked Fluorescent Assay (ELFA) on the automated Mini VIDAS® system (bioMérieux, Marcy-l'Étoile, France) according to the manufacturer's protocol. Quantitative antibody thresholds were classified as follows:

- a) Negative: < 4 IU/mL
- b) Equivocal: 4 to < 8 IU/mL
- c) Positive: ≥ 8 IU/mL

Equivocal results were re-tested; sustained equivocal values were handled per standardized diagnostic protocols.

Molecular Analysis (Reference Standard)

Conventional PCR targeting the 529-bp repetitive sequence of *T. gondii* was performed on extracted leukocyte DNA.

DNA Extraction

Target DNA was extracted from four 6-mm DBCS punches using the PureLink™ Viral RNA/DNA Mini Kit (Invitrogen, Carlsbad, CA, USA):

- a. DBCS punches were placed into a 1.5 mL microcentrifuge tube containing 25 μ L Proteinase K and 200 μ L Lysis Buffer, vortexed for 15 minutes, and incubated at 56°C for 15 minutes.
- b. Following lysis, 250 μ L absolute ethanol was added and mixed. The lysate was loaded onto a spin column and centrifuged at 6,800 $\times$ g for 1 minute.
- c. Two sequential washing steps were performed using 500 μ L Wash Buffer W5 at 6,800 $\times$ g for 1 minute each, followed by a dry centrifugation at 20,000 $\times$ g for 1 minute.
- d. Bound DNA was eluted in 30 μ L RNase-free Water (E3) after 1-minute room-temperature incubation and centrifuged at 20,000 $\times$ g for 1 minute. Eluted DNA was stored at -80° C.

Amplification Protocols and Primers

PCR reactions (25 μ L total volume) contained 12.5 μ L of 2 $\times$ DreamTaq Master Mix (Thermo Fisher Scientific; Taq DNA polymerase, dNTPs, MgCl₂), 0.4 μ M of each target primer, and 50 ng template DNA.

1. **Forward Primer (TOX-F):** 5'-CGCTGCACGGAGGAAGACGAAAGTTG-3'
2. **Reverse Primer (TOX-R):** 5'-CGCTGCACACAGTGCATCTGGATT-3'

Thermal cycling consisted of an initial denaturation at 95°C for 5 minutes; 35 cycles of denaturation at 95°C for 30 seconds, primer annealing at 58°C for 40 seconds, and extension at 72°C for 50 seconds; with a final extension at

72°C for 5 minutes. Amplicons were separated via electrophoresis on a 1.5% agarose gel containing E-Z Vision dye and visualized under UV/blue-light transillumination to confirm the 529-bp band.

Quality Assurance and Contamination Control

Physical separation was maintained between reagent preparation, specimen extraction, template addition, and post-amplification analysis rooms. Negative controls (nuclease-free water) and positive controls (*T. gondii* RH strain DNA) were included in each extraction and amplification run.

Statistical Analysis

Data were entered into Microsoft Excel and analyzed using R software (version 4.3.2). Categorical variables were expressed as absolute frequencies (n) and percentages (%). Bivariate comparisons between serological and molecular outcomes were performed using Pearson's chi-square (χ^2) test or Fisher's exact test where appropriate. Diagnostic accuracy parameters—sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall diagnostic accuracy—were calculated for IgG serology using PCR as the reference standard. Corresponding 95% confidence intervals (95% CI) were derived via the Clopper-Pearson exact binomial method (Clopper & Pearson, 1934; McGrath & Burke, 2024; Vrbín, 2023). Inter-test agreement was quantified using Cohen's kappa coefficient (κ) and interpreted according to Landis and Koch criteria (Vanbelle et al., 2024). McNemar's test was used to evaluate marginal symmetry between paired diagnostic proportions. Statistical significance was defined as $p < .005$ to minimize Type I error rates.

Ethical Considerations

The study protocol was reviewed and approved by the Ethics Committee of the School of Public Health, University of Kinshasa (Approval No.: EPS/CE/287B/2025; valid December 22, 2025 to December 22, 2026). Written informed consent was obtained from all participants prior to data and sample collection. Participant data were anonymized and stored on password-protected electronic systems.

RESULTS

Sociodemographic Characteristics

A total of 71 HIV-positive individuals with clinical features suggestive of toxoplasmosis were enrolled. Females accounted for 59.2% (n=42) of the study sample, yielding a female-to-male ratio of 1.4:1. The mean age of participants was 44±12 years (range: 18–75 years). The 36–45 age bracket was the most frequent (28.2%, n=20), followed by 46–55 years (26.8%, n=19). Secondary education was attained by 71.8% (n=51) of participants. Regarding employment, 31.0% (n=22) worked in the informal sector, while merchants and housewives comprised 18.3% (n=13) each. Geographically, participants originated primarily from Haut-Katanga (21.1%, n=15), Kongo Central (21.1%, n=15), and Maniema (16.9%, n=12). Comprehensive sociodemographic profiles are presented in Table 1.

Table 1:
Sociodemographic Characteristics of the Study Population (N=71)

Parameter	Sub-category	Frequency (n)	Percentage (%)
Sex	Female	42	59.2
	Male	29	40.8
Age Group (Years)	18–25	5	7.0
	26–35	14	19.7
	36–45	20	28.2
	46–55	19	26.8
	56–65	10	14.1
	>65	3	4.2
Education Level	Illiterate	5	7.0
	Primary	4	5.6
	Secondary	51	71.8
	Higher / University	6	8.5
	Data missing	5	7.0
Occupation	Informal employment	22	31.0
	Merchant	13	18.3
	Housewife	13	18.3
	Unemployed	8	11.3
	Engineer	4	5.6
	Police officer	4	5.6
	Military personnel	3	4.2
	Driver	2	2.8
	Student	1	1.4
	Data missing	1	1.4
Religion	Christian	47	66.2
	Muslim	14	19.7
	Non-believer	9	12.7
	Kimbanguist	1	1.4
Province of Origin	Haut-Katanga	15	21.1
	Kongo Central	15	21.1
	Maniema	12	16.9
	Kasaï	9	12.7
	Nord-Kivu	6	8.5
	Sud-Kivu	5	7.0
	Haut-Lomami	4	5.6
	Ituri	3	4.2
	Équateur	2	2.8

Diagnostic Assay Results and Inter-Method Agreement

All 71 patients were confirmed HIV-positive by reference molecular diagnostic testing. Anti-*T. gondii* IgG serology was positive in 32.4% (n=23) of cases, whereas PCR amplified *T. gondii* DNA in 39.4% (n=28) of cases (Table 2).

Table 2:
Serological (IgG) and Molecular (PCR) Detection Rates for Toxoplasmosis (N=71)

Diagnostic Assay	Classification	Frequency (n)	Percentage (%)
Anti- <i>T. gondii</i> IgG Serology	Positive	23	32.4
	Negative	48	67.6
Molecular Amplification (PCR)	Positive	28	39.4
	Negative	43	60.6

Cross-tabulation of the two testing modalities (using PCR as the reference standard) revealed 19 true positives (TP), 39 true negatives (TN), 4 false positives (FP), and 9 false negatives (FN) (Table 3). Diagnostic discordance was observed in 18.3% (n=13) of total samples. Notably, 9 patients (12.7% of the total cohort) were seronegative for anti-*T. gondii* IgG but demonstrated positive PCR detection.

Cross-tabulation yielded a statistically significant association between serology and molecular detection ($\chi^2=23.94, df=1, p<.001$; Fisher’s exact test $p<.001$). The odds ratio (OR) was 19.38 (95% CI [4.93,98.49]), indicating that IgG-seropositive individuals had 19 times higher odds of being PCR-positive compared to seronegative individuals.

Table 3:
2x2 Contingency Matrix Comparing IgG Serology Against Reference Molecular PCR

Diagnostic Test (IgG Index Test)	Reference Standard: PCR Positive (+)	Reference Standard: PCR Negative (-)	Total (n)
IgG Positive (+)	19 (TP)	4 (FP)	23
IgG Negative (-)	9 (FN)	39 (TN)	48
Total (n)	28	43	71

Note. TP = True Positive; FP = False Positive; FN = False Negative; TN = True Negative. Chi-square test: $\chi^2=23.94, df=1, p<.001$. Fisher’s exact test: $p<.001$. Odds Ratio = 19.38 (95% CI [4.93,98.49]).

Diagnostic Performance Parameters

Evaluating IgG serology against PCR as the reference method yielded a sensitivity of 67.9% (95% CI [49.3%,82.1%]) and a specificity of 90.7% (95% CI [77.9%,96.2%]). The PPV and NPV were 82.6% (95% CI [63.2%,96.2%]) and 81.3% (95% CI [68.1%,89.4%]), respectively. The overall diagnostic accuracy was 81.7% (95% CI [70.7%,89.8%]). Cohen’s kappa coefficient was calculated as $\kappa=0.60$ (95% CI [0.39,0.82]), indicating

moderate inter-test agreement. McNemar’s test yielded $p=.267$, indicating no statistically significant systematic asymmetry in discordant classifications. Performance statistics are summarized in Table 4.

Table 4:
Diagnostic Performance Parameters of Anti-*T. gondii* IgG Serology Relative to Molecular PCR

Performance Metric	Point Estimate	Expressed Percentage (%)	Exact 95% Binomial CI
Sensitivity	0.6786	67.9	49.3% – 82.1%
Specificity	0.9070	90.7	77.9% – 96.2%
Positive Predictive Value (PPV)	0.8261	82.6	63.2% – 96.2%
Negative Predictive Value (NPV)	0.8125	81.3	68.1% – 89.4%
Disease Prevalence (PCR Reference)	0.3944	39.4	28.0% – 51.8%
Detected Seroprevalence	0.3239	32.4	21.8% – 44.5%
Balanced Accuracy	0.7928	79.3	–
Overall Diagnostic Accuracy	0.8169	81.7	70.7% – 89.8%
Null Information Rate (NIR)	0.6056	60.6	–
Cohen’s Kappa Coefficient (κ)	0.6044	–	0.39 – 0.82
McNemar’s Test p-value	.2673	–	–
Accuracy vs. NIR Test (p-value)	<.0001	–	–

Note. CI = Confidence Interval; NA/ – = Not Applicable for exact binomial point estimation.

DISCUSSION

This study evaluates automated serology against molecular PCR amplification for diagnosing *Toxoplasma gondii* in people living with HIV (PLHIV) in Kinshasa, Democratic Republic of the Congo.

The observed female predominance (59.2%, female-to-male ratio 1.4:1) aligns with demographic distributions reported in HIV cohorts across Kinshasa (Booto et al., 2022; Losenga et al., 2022; Ministry of Planning and Monitoring & Ministry of Public Health [DRC], 2014) and broader sub-Saharan African contexts (Konaté et al., 2022). Social factors, gender disparities, economic vulnerabilities, and heightened biological susceptibility of mucosal tissues contribute to these trends (Gilbert et al., 2024; Jones et al., 2024). Furthermore, higher healthcare utilization rates among women, driven partly by antenatal and PMTCT screening programs, facilitate higher rate detection compared to men (Mekonnen et al., 2025; Joint United Nations Programme on HIV/ AIDS [UNAIDS], 2025).

The mean age of 44±12 years, with peak representation in the 36–45 age band (28.2%), matches demographic patterns reported in Congolese HIV studies (Bongenya et al., 2020; Booto et al., 2022). This distribution underscores the burden of HIV coinfections on economically active age groups in sub-Saharan Africa (UNAIDS, 2025). The high

proportion of participants employed in the informal economy (31.0%) aligns with economic structures in Kinshasa, where informal labor arrangements predominate (Asiani, 2024; Mamboti et al., 2025). Furthermore, the wide geographical origin of participants across several Congolese provinces reflects migration corridors toward Kinshasa, where mobility and regional social dynamics influence infection transmission and latent disease reactivation risks (Chimoy et al., 2024).

The higher detection rate by PCR (39.4%) relative to IgG serology (32.4%) highlights the utility of direct parasite DNA detection in immunocompromised patients. While serological tests rely on an intact humoral immune response, severe cellular immune impairment in advanced HIV can impair antibody production, leading to false-negative serological results despite active parasitic replication (Kim et al., 2024; Wesolowski et al., 2023). The seroprevalence observed in this study (32.4%) falls within the 30%–50% range reported among PLHIV across sub-Saharan Africa (Rostami et al., 2021; Suprianto et al., 2026; Wesolowski et al., 2023).

Despite a strong statistical association between IgG serology and PCR ($OR=19.38, p<.001$), 12.7% ($n=9$) of participants were IgG-negative but PCR-positive. This subgroup represents a clinically challenging population: severely immunosuppressed patients who fail to produce detectable anti-*T. gondii* antibodies despite active circulating parasitemia (Lindström et al., 2022). Misclassifying these patients as uninfected based solely on negative serology risks delaying necessary antiparasitic therapy. Conversely, 4 patients (5.6%) were IgG-positive but PCR-negative. This finding likely reflects past, latent infection with encysted bradyzoites without active tissue lysis or circulating DNA, or parasitic DNA levels below the assay's limit of detection (Kim et al., 2024; NIH, 2025).

The moderate sensitivity of IgG serology (67.9%) relative to PCR confirms that serological screening alone is insufficient to exclude active toxoplasmosis in advanced HIV infection (Wesolowski et al., 2023). However, its high specificity (90.7%) and PPV (82.6%) indicate that a positive IgG result in a symptomatic patient strongly supports exposure to the parasite, providing diagnostic value when molecular testing is unavailable (Kim et al., 2024). Overall

accuracy (81.7%) and inter-test agreement ($\kappa=0.60$) reflect moderate concordance (Vanbelle et al., 2024), demonstrating that while serology provides useful initial screening data, it cannot replace molecular amplification when confirming active clinical disease (Guitard et al., 2024; Souza et al., 2023; Suprianto et al., 2026).

Implementing routine PCR testing in resource-limited settings like Kinshasa remains challenging due to instrument costs, reagent supply lines, and technical infrastructure demands (Guarche-Ibañez et al., 2022). Nevertheless, given the morbidity of toxoplasmic encephalitis, expanding access to molecular assays or point-of-care nucleic acid platforms is essential for improving clinical management in tertiary centers. Combined diagnostic algorithms that integrate clinical assessment, serology, and molecular testing offer a balanced approach to mitigating diagnostic misclassification.

CONCLUSION

This study demonstrates that conventional PCR targeting the 529-bp repetitive element identifies a higher rate of *Toxoplasma gondii* positivity (39.4%) than IgG automated serology (32.4%) among PLHIV presenting with suspected active toxoplasmosis in Kinshasa. While inter-test agreement was moderate ($\kappa=0.60$), the presence of seronegative/PCR-positive cases underscores the limitations of relying solely on humoral antibody detection in immunocompromised individuals. Combined diagnostic strategies integrating serological screening and confirmatory molecular testing are recommended to improve early detection and therapeutic outcomes in resource-constrained health systems.

Limitations

- Sampling Method and Size:** The use of a non-probability convenience sample ($N=71$) from selected referral centers in Kinshasa introduces potential selection bias and restricts generalizability to the broader population of PLHIV in the DRC.
- Cross-Sectional Design:** The single-time-point assessment prevents establishing temporal relationships between infection acquisition, seroconversion, and clinical progression, limiting

the ability to differentiate recent primary infection from acute reactivation.

3. **Missing Immunological Markers:** Absolute CD4+ T-cell counts and plasma HIV RNA viral loads were not systematically measured, preventing direct stratification of diagnostic performance across specific tiers of host immunosuppression.
4. **Reference Standard Constraints:** Conventional PCR was evaluated as the primary reference standard without routine parallel quantitative real-time PCR or advanced neuroimaging (e.g., MRI/CT), which may affect the classification of isolated serological findings.

Recommendations

To the World Health Organization (WHO) and Global Health Partners

- A. Support the validation and deployment of accessible, low-cost, portable PCR platforms and point-of-care molecular assays adapted for low-resource clinical settings in sub-Saharan Africa.
- B. Update regional clinical guidelines to emphasize integrated diagnostic algorithms combining serological screening with targeted molecular testing in HIV care.

To the Ministry of Public Health, Hygiene and Social Welfare of the DRC

- I. Strengthen national laboratory networks by improving access to molecular diagnostic equipment and standardized reagents in regional referral hospitals.
- II. Conduct training programs for laboratory personnel focusing on quality control and standard operating procedures for molecular diagnostic platforms.
- III. Enhance decentralized epidemiological surveillance networks to track opportunistic co-infections among PLHIV.

To the Scientific and Research Community

- I. Conduct prospective, multi-center cohort studies with larger sample sizes to evaluate the

longitudinal prognostic value of discordant serological and molecular findings.

- II. Investigate the impact of host immune recovery (CD4+ count and viral suppression trends) on diagnostic test performance.
- III. Perform genomic characterization of circulating *T. gondii* strains in Central Africa to assess variations in target sequences and diagnostic assay sensitivity.
- IV. Conduct cost-effectiveness analyses comparing routine serological screening with targeted PCR testing strategies in resource-limited settings.

List of Abbreviations

1. **ART:** Antiretroviral Therapy
2. **CI:** Confidence Interval
3. **DBCS:** Dried Buffy Coat Spots
4. **DBS:** Dried Blood Spot
5. **DRC:** Democratic Republic of the Congo
6. **EDTA:** Ethylenediaminetetraacetic Acid
7. **ELFA:** Enzyme-Linked Fluorescent Assay
8. **FN:** False Negative
9. **FP:** False Positive
10. **IgG / IgM:** Immunoglobulin G / Immunoglobulin M
11. **NIH:** National Institutes of Health
12. **NIR:** Null Information Rate
13. **NPV:** Negative Predictive Value
14. **OR:** Odds Ratio
15. **PCR:** Polymerase Chain Reaction
16. **PLHIV:** People Living with HIV
17. **PPV:** Positive Predictive Value
18. **TN:** True Negative
19. **TP:** True Positive
20. **UNAIDS:** Joint United Nations Programme on HIV / AIDS
21. **WHO:** World Health Organization

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Use of Artificial Intelligence (AI) Declaration: The authors declare that no artificial intelligence (AI) or AI-assisted technologies were used in the study design, data collection, data analysis, or preparation of this manuscript.

Authors' Contributions:

- i. **Y. L. Malumbi:** Conceptualization, Methodology, Investigation, Writing – original draft.
- ii. **G. Booto Ipay:** Data curation, Formal analysis, Validation, Visualization, Writing – review & editing.
- iii. **L. Losenga Omombo:** Investigation, Writing – review & editing.
- iv. **C. Tshinguta:** Investigation, Validation.
- v. **B. Bongonya:** Investigation, Validation.
- vi. **E. N. Kamangu:** Conceptualization, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

Data Availability Statement: The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable academic request.

Ethical Approval: This study was approved by the Institutional Ethics Committee of the School of Public Health, University of Kinshasa, Democratic Republic of the Congo (Reference No.: EPS/CE/287B/2025; issued December 22, 2025; valid through December 22, 2026). All procedures performed involving human participants adhered to the ethical standards of the institutional research committee and the 1964 Declaration of Helsinki and its later amendments. Anonymity and data confidentiality were strictly maintained.

Informed Consent and anonymity: Written informed consent was obtained from all individual participants included in the study prior to blood sample collection and questionnaire administration.

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