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Enhanced Detection of Early HIV Infections Using a Combined Diagnostic Algorithm in the Central African Republic: A Cross-Sectional Diagnostic Accuracy Study

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Abstract

Background: Human immunodeficiency virus (HIV) infection remains a major global public health challenge, particularly in resource-limited settings such as the Central African Republic. Early and accurate diagnosis is essential to ensure timely initiation of antiretroviral therapy and to reduce ongoing transmission. However, conventional serological assays may fail to detect recent infections during the acute phase due to the diagnostic window period. This study evaluated the performance of an optimized HIV screening algorithm combining a fourth-generation enzyme-linked immunosorbent assay (ELISA), confirmatory rapid diagnostic tests, and HIV-1 polymerase chain reaction (PCR) for discordant cases, compared with a standard serological algorithm. **Methods:** A descriptive and analytical cross-sectional study was conducted over nine months (December 2024-August 2025) at the National Laboratory of Clinical Biology and Public Health in Bangui. A total of 500 participants aged 18 months and older were included using exhaustive sampling. Initial screening was performed using a fourth-generation ELISA. Reactive samples were confirmed using Determine™ and Uni-Gold™ rapid diagnostic tests. Discordant results were further investigated using HIV-1 PCR for viral RNA detection. Diagnostic performance was assessed by calculating sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). Diagnostic gain and reduction in median diagnostic delay were also evaluated. **Results:** Among the 500 participants, 120 (24.0%) were reactive by fourth-generation ELISA. Serological confirmation identified 25 discordant cases (20.8%). HIV-1 RNA was detected in 18 of these cases (72.0%), indicating early HIV infection. The optimized algorithm confirmed 113 HIV infections (22.6%), compared with 95 cases identified using the standard algorithm. The median diagnostic delay decreased from 28 to 14 days. Overall, the optimized algorithm achieved a sensitivity of 98.2% and a specificity of 99.2%, with an 18.9% increase in case detection compared with the standard approach. **Conclusions:** The optimized HIV screening algorithm improves early and reliable detection of HIV infection, particularly during the acute phase, by reducing false-negative results and shortening diagnostic delay. Its implementation in resource-limited settings may enhance early case detection, improve patient management, and strengthen public health strategies for HIV control.

Keywords

HIV-1, Early Diagnosis, Fourth-Generation ELISA, HIV-1 PCR, Diagnostic Algorithm, Resource-Limited Settings

1. Introduction

More than forty years after its discovery, infection with the human immunodeficiency virus (HIV) remains a major global public health challenge. This retrovirus causes acquired immunodeficiency syndrome (AIDS), characterized by the progressive destruction of $CD4^+$ T lymphocytes, leading to increased susceptibility to opportunistic infections and certain malignancies [1] [2].

HIV is an enveloped virus belonging to the family *Retroviridae*, genus *Lentivirus* [3]. Its structure includes an envelope containing glycoproteins gp120 and gp41, a p24 capsid, and a genome composed of two copies of single-stranded RNA encoding *gag*, *pol*, *env*, and regulatory genes, as well as essential enzymes such as reverse transcriptase, integrase, and protease [4] [5].

Two main types are recognized: HIV-1, responsible for the global pandemic, and HIV-2, largely confined to West Africa and characterized by slower disease progression [6]. HIV-1 exhibits extensive genetic diversity, dominated by group M and its subtypes, which complicates diagnosis, epidemiological surveillance, and treatment strategies [7] [8].

In 2023, approximately 39 million people were living with HIV worldwide, with more than one million new infections, mainly in sub-Saharan Africa [9] [10]. In the Central African Republic, adult HIV prevalence remains high (3% - 4%) [11], and access to molecular diagnostics is limited, with testing relying primarily on rapid serological assays. Optimizing HIV screening strategies is therefore critical to improving patient care and reducing transmission.

HIV infection follows a well-defined biomarker kinetics: viral RNA becomes detectable during the first 1 - 2 weeks, p24 antigen between weeks 2 and 4, and HIV-specific antibodies from weeks 3 to 6 onward [12]-[14]. This sequence determines the performance and limitations of diagnostic tests.

World Health Organization (WHO) algorithms rely mainly on sequential rapid diagnostic tests [15]. Although effective for expanding access to testing, these algorithms lack sensitivity during acute infection, generate discordant results, and do not always account for biomarker kinetics [16]-[18]. The absence of systematic RNA detection may delay diagnosis and early treatment initiation.

We hypothesized that an optimized algorithm based on biomarker kinetics, combining a fourth-generation ELISA, confirmatory rapid tests (Determine^{TM} and Uni-Gold^{TM}), and targeted PCR for discordant results, would improve the timeliness and reliability of HIV diagnosis.

The overall objective of this study was to evaluate the performance of this optimized algorithm in a resource-limited setting in order to improve early HIV diagnosis and patient management in the Central African Republic.

2. Methods

2.1. Study Setting

The study was conducted at the National Laboratory of Clinical Biology and Public Health in Bangui, Central African Republic, the main reference center for HIV diagnosis in the country. The laboratory is equipped to perform serological testing, fourth-generation ELISA, rapid diagnostic tests, and HIV-1 PCR, enabling full implementation of the diagnostic algorithm under study.

2.2. Study Design and Period

This was a descriptive and analytical cross-sectional study conducted over nine months, from December 2024 to August 2025, aimed at evaluating the performance of an optimized HIV screening algorithm.

2.3. Study Population

The target population included individuals aged 18 months and older presenting for HIV testing, with or without clinical symptoms suggestive of infection.

2.4. Inclusion Criteria

Participants (or parents/legal guardians for minors) who provided informed consent, agreed to blood sample collection, and were not receiving antiretroviral therapy were included.

2.5. Exclusion Criteria

Individuals who refused participation, those with insufficient biological samples, and patients already diagnosed with HIV and receiving antiretroviral therapy were excluded.

2.6. Sampling

An exhaustive sampling strategy was used, including all eligible participants presenting during the study period, to obtain a comprehensive assessment of algorithm performance.

2.7. Laboratory Methods

Biological samples consisted of whole blood collected in EDTA and plain tubes. Serological testing included Determine^{TM} and Uni-Gold^{TM} rapid tests and a fourth-generation ELISA. HIV-1 PCR was performed for discordant or indeterminate cases. All tests were conducted according to standardized laboratory protocols and WHO recommendations.

2.8. Ethical Approval Statement

Ethical Approval and Consent to Participate

The present study was reviewed and approved by the Doctoral School of Science and Technology of the University of Bangui (EDST).

All procedures were conducted in accordance with the applicable national ethical standards as well as the international principles outlined in the Declaration of Helsinki of the World Medical Association (2013 revision).

The study adhered to the fundamental principles of biomedical research ethics, including respect for human dignity, participant autonomy, beneficence, non-maleficence, and justice.

Consent to Participate

Written informed consent was obtained from all participants prior to their inclusion in the study.

Before signing the consent form, participants received clear and detailed information regarding:

- the objectives of the study;
- the procedures involved;
- the potential benefits and risks;
- the confidentiality of the collected information;
- their right to refuse participation or to withdraw from the study at any time without any consequences for their medical care.

For minors or legally incapacitated participants, consent was obtained from their legal representatives in accordance with applicable ethical requirements.

Confidentiality and Data Protection

The collected data were anonymized using unique identification codes. No directly identifiable participant information was included in the analytical databases.

Access to the data was strictly limited to authorized members of the research team. Electronic data were password-protected, and physical documents were stored in a secure location.

2.9. Data Collection and Analysis

Data were collected using standardized forms. Statistical analysis was performed using EPI Info version 7. Results were presented as frequencies, percentages, means, and standard deviations. Algorithm performance was evaluated using sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), with statistical significance set at $p < 0.05$.

3. Results

3.1. Study Population and Participant Flow

During the study period, a total of 500 individuals underwent HIV screening at the National Laboratory of Clinical Biology and Public Health in Bangui. Of these, 120 participants (24.0%) tested reactive using the fourth-generation ELISA, while 380 (76.0%) were non-reactive.

All ELISA-reactive individuals were included in the diagnostic accuracy analysis. Among ELISA-negative participants, 90 individuals (23.7%) reported recent HIV exposure within the previous six weeks and were scheduled for follow-up testing according to the study protocol. However, follow-up results were not available at the time of analysis and were therefore not included in the final evaluation.

The flow of participants and distribution of ELISA results are summarized in Table 1 and Table 2.

3.2. Reference Standard

Diagnostic accuracy was assessed using a **composite reference standard**, defined as follows:

- concordant positive rapid diagnostic test results (DetermineTM and Uni-Gold^{TM}), or
- HIV-1 PCR positivity in cases with discordant serological results

Individuals with negative PCR results in discordant cases were classified as uninfected.

3.3. Characteristics of the Study Population

The study population was predominantly female (58.0%), with the largest age group being 25 - 34 years (37.0%). A recent risk exposure (<6 weeks) was reported in 36.0% of participants, indicating a substantial proportion at risk of early infection.

Sociodemographic and clinical characteristics of participants are summarized in Table 1.

The population was predominantly female (58%), with a median age in the 25 - 34-year group. Recent risk exposure was reported by more than one-third of participants (36%), justifying the use of diagnostic tools capable of detecting very early infections.

Table 1. Sociodemographic and clinical characteristics of the study population (n = 500).

Characteristic	Number (n)	Percentage (%)
Sex		
Female	290	58.0
Male	210	42.0
Age (years)		
<25	120	24.0
25 - 34	185	37.0
35 - 44	125	25.0
≥45	70	14.0
Recent risk exposure (<6 weeks)		
Yes	180	36.0
No	320	64.0

3.4. Initial ELISA Screening

Among the 500 participants, 120 (24.0%) were ELISA reactive, while 380 (76.0%) were non-reactive (Table 2). Among ELISA-negative individuals, 90 (23.7%) reported recent exposure and were scheduled for repeat testing.

Fourth-generation HIV ELISA results

The results of the initial fourth-generation ELISA screening are presented in Table 2.

Table 2. Initial screening results by fourth-generation HIV ELISA.

Fourth-generation ELISA result	Number (n)	Percentage (%)
Negative	380	76.0
Positive	120	24.0
Total	**500**	**100**

Among the 380 ELISA-negative patients, 90 (23.7%) reported recent exposure and were scheduled for retesting after 2 - 4 weeks.

3.5. Sequential Confirmation by Determine™/Uni-Gold™ Rapid Tests

Serological confirmation using rapid diagnostic tests

Among the 120 ELISA-reactive individuals, 95 (79.2%) had concordant positive results on Determine^{TM} and Uni-Gold^{TM}, confirming HIV infection. However, 25 cases (20.8%) showed discordant or indeterminate results (Table 3).

3.6. HIV-1 PCR Results in Discordant Cases

Among the 25 discordant cases, HIV-1 RNA was detected in 18 individuals (72.0%), while 7 (28.0%) had undetectable viral RNA (Table 4).

These PCR-positive cases were classified as confirmed HIV infections, likely corresponding to early-stage infections not fully detectable by serological assays.

The serological confirmation results obtained using rapid diagnostic tests are shown in Table 3.

Table 3. Serological confirmation results.

Rapid test results	Number (n)	Percentage (%)
Determine+/Uni-Gold+ (concordant positive)	95	79.2
Discordant/indeterminate results	25	20.8
Total	**120**	**100**

These findings show that nearly one in five patients had serological discordance, highlighting the limitations of serological confirmation alone.

3.7. Added Value of HIV-1 PCR in Discordant Cases

The distribution of HIV-1 PCR results among discordant cases is detailed in Table 4.

Table 4. HIV-1 PCR results among discordant patients (n = 25).

HIV-1 PCR result	Number (n)	Percentage (%)
HIV RNA detected	18	72.0
HIV RNA not detected	7	28.0
Total	**25**	**100**

Seventy-two percent of discordant cases corresponded to very early HIV infections detectable only by PCR.

3.8. Final HIV Diagnosis Using the Optimized Algorithm

After applying the complete diagnostic algorithm, a total of 113 individuals (22.6%) were classified as HIV-positive, while 387 (77.4%) were considered HIV-negative (Table 5).

Among confirmed infections, 18 cases (15.9%) were identified exclusively through PCR among discordant samples and would have been missed using a purely serological algorithm.

Table 5. Final HIV status after full application of the algorithm.

Final HIV status	Number (n)	Percentage (%)
Confirmed HIV infection	113	22.6
HIV not detected/not confirmed	387	77.4
Total	**500**	**100**

Among confirmed cases, 18 (15.9%) were very early infections that would have been missed by a strictly serological algorithm.

3.9. Comparison with the Standard Serological Algorithm

Compared to the standard algorithm based solely on rapid diagnostic tests, the optimized approach detected more HIV cases (113 vs. 95) and substantially reduced the number of false-negative results (2 vs. 18).

The estimated median diagnostic delay was reduced from 28 days to 14 days (Table 6). Overall, this corresponds to a relative increase in case detection of 18.9%.

Table 6. Comparison of diagnostic performance.

Indicator	Standard algorithm	Optimized algorithm
HIV cases detected	95	113
False-negative cases	18	2
Median diagnostic delay (days)	28	14

The optimized algorithm yielded a relative detection increase of 18.9%.

3.10. Diagnostic Performance

The diagnostic performance of the optimized algorithm was assessed using a contingency table (Table 7).

- True positives: 111
- False positives: 3
- True negatives: 384
- False negatives: 2

These results indicate a high diagnostic performance of the optimized algorithm.

Table 7. Performance indicators of the optimized algorithm.

Indicator	Value
Sensitivity	98.2% (95% CI: 93.7 - 99.8)
Specificity	99.2% (95% CI: 97.8 - 99.9)
Positive predictive value (PPV)	97.4%
Negative predictive value (NPV)	99.5%

Continued

Overall accuracy	99.0%
Positive likelihood ratio (LR+)	122.7
Negative likelihood ratio (LR−)	0.018

Although the optimized algorithm demonstrated high sensitivity and specificity, these findings should be interpreted with caution due to the use of a composite reference standard and the absence of systematic PCR testing in all participants.

3.11. Study Limitations

This study has several limitations. Its single-centre design may limit generalisability. PCR was performed only in discordant cases, introducing potential verification bias. Missing follow-up data in ELISA-negative participants may underestimate early infections. The composite reference standard may lead to misclassification, and no cost-effectiveness or feasibility analysis was conducted.

4. Discussion

In this study, the optimized HIV screening algorithm combining a fourth-generation ELISA, rapid diagnostic tests, and HIV-1 PCR demonstrated excellent diagnostic performance, with a sensitivity of 98.2% and a specificity of 99.2%.

The observed negative predictive value was particularly high (99.5%), highlighting the reliability of the algorithm for ruling out infection in a high-prevalence, resource-limited setting. Importantly, the integration of HIV-1 PCR allowed the identification of 72% of discordant cases, most of which likely correspond to early-stage infections that would not have been detected by a purely serological approach.

These findings are consistent with previous evidence showing the limitations of rapid and serological assays in early HIV infection. A systematic review and meta-analysis including 18 studies (approximately 110,000 test results) reported an overall sensitivity of 94.5% for rapid HIV tests compared with fourth-generation enzyme immunoassays and 93.7% compared with nucleic acid amplification tests (NAAT), with performance variability depending on the epidemiological context and country income level [19]. These data support the notion that although rapid tests are essential for expanding access to diagnosis, their sensitivity remains suboptimal for detecting acute infections.

Similarly, Helleberg *et al.* (2023), in a study conducted in Eswatini, evaluated the Determine™ HIV Early Detect rapid test and reported a very low sensitivity (20%) for acute HIV infection despite excellent specificity (99.8%) [20]. This marked discrepancy highlights the persistent diagnostic gap during the early phase of infection, which is also reflected in our findings where serological discordance was frequent.

Moyo *et al.* (2018) further demonstrated that the sensitivity of fourth-generation rapid tests such as Determine and AQ+ varies substantially in pre-seroconversion stages, ranging from 66.7% to 81.5% [21]. These results suggest that even assays labeled as "fourth-generation" may fail to reliably detect early infection, particularly in high-incidence populations where early diagnosis is critical for transmission prevention.

In addition, data from an African context, specifically a study conducted in Gabon among blood donors, reported sensitivities ranging from 65.6% to 90.9% across different rapid tests when compared with fourth-generation ELISA [22]. These findings reinforce the notion that while rapid diagnostic tests offer high specificity, their sensitivity remains insufficient for early infection detection, leading to potential missed cases if used as standalone diagnostic tools.

Taken together, the evidence from the literature strongly supports our results. While rapid diagnostic tests remain indispensable for expanding HIV testing coverage, they cannot replace combined serological and molecular strategies when the objective is early detection of infection. In our study, the addition of HIV-1 PCR effectively bridged the diagnostic window period not covered by serological assays, resulting in a significant reduction in false-negative results and an improvement in early case detection.

From a public health perspective, these findings are particularly relevant in resource-limited settings such as the Central African Republic, where delayed diagnosis contributes to ongoing transmission. The optimized algorithm therefore represents a pragmatic approach that balances feasibility with improved diagnostic accuracy, particularly for identifying early infections that are critical for prevention strategies and timely treatment initiation.

5. Conclusions

This study demonstrates that an optimized HIV screening algorithm combining a fourth-generation ELISA, rapid diagnostic tests, and targeted HIV-1 PCR for discordant cases provides improved diagnostic performance compared with a standard serological approach. The incorporation of PCR enabled the identification of early HIV infections that would have been missed by serology alone, thereby reducing false-negative results and improving overall case detection.

The findings highlight the added value of integrating molecular testing into HIV diagnostic strategies, particularly for the detection of recent infections within the diagnostic window period. This approach also contributes to a reduction in diagnostic delay and improves the accuracy of HIV diagnosis.

In resource-limited settings such as the Central African Republic, where access to molecular diagnostics remains restricted, the implementation of a combined algorithm represents a pragmatic and effective strategy to enhance early HIV detection. Such an approach could strengthen HIV screening programs, support earlier initiation of antiretroviral therapy, and ultimately contribute to improved public health outcomes and reduced transmission.

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Conflicts of Interest

The authors declare no financial or non-financial conflicts of interest related to this study.

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