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Updates on the Clinical Epidemiology of HIV-1 Group O Strains in Cameroon and Potential Implications on Diagnosis and Treatment Strategies

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Cameroon is an epicenter of diverse HIV-1 strains, with diagnostic and management challenges. The objective herein was to update HIV-1 non-M prevalence and compare diagnostic performance of two- versus three-test algorithms. A facility-based study was conducted in February 2024 on 2207 HIV-1 clinical samples at the Chantal Biya International Reference Centre (Yaoundé, Cameroon). HIV-1 non-M were identified by molecular phylogeny. Rapid diagnostic tests (RDTs) used in the two-test (Determine and KHB colloidal gold) versus three-test (First Response, One Step, and KHB) algorithms were evaluated on non-M, with ACRO (HIV1/2 and p24) as independent RDT. No group N (0%) nor P (0%) was found, whereas nine group O were identified (0.4%; 95% CI, 0.2%—0.8%). For individuals harboring group O (mean age, 43 ± 12 years; 50% female), median (IQR): duration since HIV diagnosis was 627 (423 to 775) weeks; viremia, 12,385 (5340 to 72,682) copies/mL; and CD4 count, 52 (39 to 228) cells/mm^3. One Step, KHB, and ACRO detected eight of eight group O (100%); First Response HIV1-2.0, seven of eight (87.5%); and Determine HIV1/2, six of eight (75%), $P = 1.00$. In this Cameroonian setting, HIV-1 group N and P are scarce, whereas group O remains low (<1%). Transitioning from the two-test (75% performance) to the three-test algorithm (87.5% performance) could lead to improved diagnostic performance on currently circulating HIV-1 group O, calling for updates in

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RDTs to adapt to viral dynamics. (*J Mol Diagn 2026, 28: 160—169; https://doi.org/10.1016/j.jmoldx.2025.10.008*)

With >40 million people living with HIV (PWH) worldwide, HIV/AIDS remains a global public health priority, especially in sub-Saharan Africa, which harbors approximately two-thirds of the global burden. West and Central Africa is significantly affected, with 5.2 million PWH and 120,000 HIV-related deaths in 2024 (*https://www.unaids.org/en/resources/fact-sheet*, last accessed July 19, 2025). With scale-up of antiretroviral therapy and improved therapeutic strategies, significant progress has been made toward attaining the Joint United Nations Program on HIV/AIDS 95-95-95 objectives. In 2024, 87% of PWH knew their status, 89% of whom were on antiretroviral therapy, and 94% of those treated were virally suppressed (*https://www.unaids.org/en/resources/fact-sheet*, last accessed July 19, 2025). Although encouraging, the most significant gap remains within the first 95 concerning diagnosis. Indeed, the last two indicators depend on the first, and therefore efforts toward achieving this should be given major priority.[1] In West and Central Africa, 80% of PWH knew their status in 2024, among the lowest globally (*https://www.who.int/teams/global-hiv-hepatitis-and-stis-programmes/hiv/strategic-information/hiv-data-and-statistics*, last accessed July 19, 2025), necessitating increased efforts in attaining testing coverage. With declining treatment-adjusted HIV prevalences and national HIV testing services positivity, the World Health Organization (WHO) encourages countries with HIV prevalence <5% currently using two consecutive reactive tests for an HIV-positive diagnosis to move to using an algorithm consisting of three consecutive reactive tests, to ensure that of all persons classified as having HIV, ≥99% will be truly living with HIV (known as the testing positive predictive value; *https://www.who.int/publications/i/item/WHO-CDS-HIV-19.34*, last accessed July 19, 2025). The use of highly sensitive and specific tests [either rapid diagnostic tests (RDTs) or enzyme immunoassays], prequalified by WHO is crucial, for accurate classification of tested individuals.

As the epicenter of HIV/AIDS, the west/central African region (and Cameroon in particular) has high HIV genetic diversity (harboring all HIV types, groups, and subtypes),[2–6] which could influence diagnostic accuracy and possibly treatment outcomes in this context still lacking individualized management of PWH.[7,8] Of note, groups N, P, and O have all been reported, with a declining trend of group O, from 1.1% in 2006 to 0.6% in 2013.[4,9] Aghokeng et al,[10] in 2009, showed that approximately 80% of PWH with group O do not receive antiretroviral therapy because of inaccuracies in diagnosis linked to low sensitivities of RDTs. Although with the continuous improvement of enzyme-linked immunosorbent assays, there is reduced risk of failure to detect group O infection, diagnostic failures are still continuously reported, especially with RDTs that do not include group O—specific antigen.[11,12] As concerns treatment, group O naturally carries a cysteine at position 181 of the reverse transcriptase (predominantly the H strains), causing natural resistance to first-generation nonnucleoside reverse-transcriptase inhibitors (NNRTIs), which were the mainstay of first-line therapy over many years in Cameroon and similar settings.[13] In the current era of dolutegravir-based regimens, this challenge should be overcome if correctly diagnosed for early and proper management (*https://www.who.int/news/item/16-07-2021-who-publishes-new-consolidated-hiv-guidelines-for-prevention-treatment-service-delivery-monitoring*, last accessed July 19, 2025). Although some evidence exists on the circulation of nongroup M strains, recent updates remain scarce (latest published in 2017 from rural southern parts of Cameroon),[14] in a setting with a broad HIV genetic diversity and a dynamic molecular epidemiology that could impact programmatic interventions at individual and population levels. Furthermore, in the context of transition from the two-test to three-test diagnostic algorithm, as recommended by the WHO (2.7% national prevalence), the evaluation of the new three-test algorithm is crucial to ensure accurate and timely detection of all circulating HIV strains and improve the chances of eliminating HIV/AIDS as a public health threat by 2030. Taking into consideration these points raised, the current study is of added value as it includes samples from all over the national territory up until 2023, therefore contributing updated information on the clinical epidemiology of these non-M strains. More importantly, this study evaluates tests used in the previous two-test algorithm, compared with the incoming three-test algorithm so as to inform national HIV testing strategies. The objectives therefore of this study were to update the prevalence of HIV-1 groups N, O, and P, and to compare the diagnostic performance of the two-test versus three-test algorithm on the detection of circulating HIV-1 nongroup-M viruses.

Materials and Methods

Study Design and Site

A facility-based cross-sectional study was conducted at the Chantal Biya International Reference Centre for Research on HIV Prevention and Management (CIRCB; Yaoundé,

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Cameroon), on clinical samples referred for genotypic resistance testing at CIRCB and stored in the CIRCB-Biobank from 2006 up until 2023. The CIRCB is a WHO HIV Drug Resistance Network national laboratory and is a large-scale reference center for clinical research in West and Central Africa aimed at optimizing diagnostic and treatment strategies of viruses of public health importance or potential in Africa.

Procedures

Using the CIRCB Antiviral Resistance electronic database, all records with HIV-1 non-M strains, up until December 2023, were searched for. These consisted of records of individuals previously diagnosed and followed up at clinical centers (diagnostic methods and clinical status at diagnosis undocumented) all over the country and received for HIV drug resistance testing at CIRCB during the study period. All individuals harboring nongroup M strains were selected, and their samples were retrieved from the CIRCB-Biobank for serologic testing. Each sample had at least two cryotubes (1 mL each) stored as backup samples, and so the samples used for further analyses had not been through any freeze-thaw cycles.

Bioinformatics Analysis

HIV-1 sequences, obtained from HIV-1 *pol* (protease and reverse transcriptase) sequencing at CIRCB using an in-house genotyping protocol (previously described),[15] were retrieved from the CIRCB Antiviral Resistance database for molecular phylogeny to confirm viral strains (*https://www.ncbi.nlm.nih.gov/nucleotide*; accession numbers PQ893190, PQ893192, PQ893193, PQ893194, PQ893195, PQ893196, PQ893197, and PQ893198). Reference sequences of pure subtypes, circulating recombinant forms (CRFs), and non-M groups in West and Central Africa were downloaded from GenBank and included in the analysis with at least three reference sequences per subtype/CRF/group. Sequence alignment was performed using MAFFT version 7.526,[16] phylogenetic tree inference was performed using maximum likelihood method on IQ-TREE version 2.0.7,[17] and tree visualization and editing were performed using Figtree version 1.4.4 (*http://tree.bio.ed.ac.uk/software/figtree*). The classification of nongroup M strains was evaluated using rapid subtyping tools, used routinely at CIRCB.[18] The tools used were Stanford HIVdb version 9.4.1,[19] COMET HIV-1 version 2.4,[20] Rega HIV subtyping tool version 3.42,[21] Geno2pheno (subtyping),[22] and Basic Local Alignment Search Tool (*https://www.hiv.lanl.gov/content/sequence/BASIC_BLAST/basic_blast.html*). Genotypic profiles of all group O sequences were also analyzed using Stanford HIVdb version 9.4.1.

Serologic Testing on HIV-1 Nongroup M

Rapid diagnostic tests were performed following manufacturer's instructions, using WHO prequalified tests in the two-test[23] and three-test algorithm (ministerial decision: number 0098/D/MINSANTE, *https://cnls.cm/catdocument/rapports-annuels*, last accessed October 23, 2025). The two-test algorithm has as first test: Determine HIV1/2 (Abbott Diagnostic Division, Hoofddorp, the Netherlands), sensitive in detecting HIV-1, HIV-2, and HIV-1 group O, according to the manufacturer; and as second test: Diagnostic kit for HIV (1+2) antibody (colloidal gold, V2 KHB; Shangai Kehua Bio-Engineering Co, Ltd, Shangai, China), sensitive in detecting HIV-1, HIV-2, and HIV-1 group O, as specified by the manufacturer. The tests in the three-test algorithm are as first test: First Response HIV1-2.0 Card Test (Premier Medical Corp. Private Limited, Gujarat, India); second test: One Step Anti-HIV (1 and 2) Test (InTec, Xiamen, China); and third test: Diagnostic kit for HIV (1+2) antibody (colloidal gold) V2 KHB (Shangai Kehua Bio-Engineering Co, Ltd), all also sensitive in detecting HIV-1, HIV-2, and HIV-1 group O, as specified by the manufacturers. An additional internal test was added during laboratory analysis, to increase sensitivity, especially in detecting early infections (ACRO Rapid Test HIV1/2 and p24; Acro Biotech Inc., Montclair, CA). The reactive biomarkers were characterized using INNO-LIA HIV I/II Score (Fujirebio Europe N.V., Ghent, Belgium), as per the manufacturer's instructions. All RDTs were performed by trained technicians at CIRCB, repeated in pairs for each test, and results were reported by six individuals independently, with the consensus used as the result. The results of INNO-LIA were interpreted by a team composing of the laboratory head (J.F.) and three senior laboratory scientists (D.A.T.K., O.E.G.B.A., and S.C.D.N.).

Interpretation of Results

Test performance was determined as the capacity of the test to render a positive result on a given sample. The sensitivity of each RDT in detecting HIV-1 nongroup M strains was determined by the number of reactive tests on the total number of samples submitted to the test. The detection sensitivity was compared between the two- and three-test algorithms.

Statistical Analysis

Epi info version 7.2.6 statistical software standalone version (*https://www.cdc.gov/epiinfo*) was used for statistical analysis. The mean, median, SD, and interquartile range (IQR) were used for nonnominal variables. The *t*-test and χ^2/Fisher exact test were used to compare continuous and categorical variables, respectively (where necessary for the entire population), with $P \leq 0.05$ considered statistically significant. Using

Table 1 Characteristics of Individuals With HIV-1 Group O

ID	Age, years	Sex	Date of diagnosis*	Region of residence	Date of GRT phlebotomy*	Last viremia, copies/mL	Last CD4, cell/mm^3
GO-CAM-20	64	Male	16/12/1999	Centre	4/8/2021	1431	46
GO-CAM-62	45	Female	1/1/2005	West	4/1/2018	5235	443
GO-CAM-95	45	Male	1/1/2014	Centre	22/10/2018	138,410	57
GO-CAM-75	26	Female	1/1/2008	West	14/8/2018	58,900	39
GO-CAM-020	35	Male	10/4/2006	Centre	14/5/2019	5446	228
GO-CAM-29	53	Male	1/6/2005	Centre	11/1/2022	86,464	U/A
GO-CAM-94	34	Female	1/1/2011	Centre	11/1/2022	18,456	U/A
GO-CAM-02	40	Female	26/7/2016	East	4/2/2022	6314	28
GO-CAM-83	55	Male	In 2010	Centre	15/9/2022	U/A	U/A

*Dates are given as day/month/year.
GRT, genotypic resistance test; ID, identification; U/A, unavailable.

prevalences of HIV-1 group O over the years as obtained from previous studies[4,24,25], a trend graph was prepared using GraphPad Prism version 8.0.1 (GraphPad Software, San Diego, CA).

Ethical Considerations

The study was conducted in accordance with the Declaration of Helsinki.[26] Samples used from the CIRCB Biobank that had previous ethical clearance for use from the ethical review board of the Faculty of Medicine and Biomedical Sciences received an authorization from the national ethics committee (reference number: 0060/UY1/FMSB/VDRC/CSD). All sample data were handled with strict confidentiality and anonymity to protect the privacy and identity of participants.

Results

Study Population

A total of 2207 records were analyzed from the CIRCB Antiviral Resistance database, with median (IQR) age of 38 (19 to 47) years, 67.8% (n = 1496) of whom were female, coming from all 10 regions of the country: 44.9% (n = 990) from the Centre region, 14.9% (n = 329) from the Littoral region, 12.9% (n = 285) from the North West region, 9.6% (n = 212) from the West region, 6.4% (n = 140) from the South West region, 4.7% (n = 103) from the East region, 2.6% (n = 57) from the North region, 2.2% (n = 49) from the extreme North region, 1.8% (n = 39) from the South region, and 0.1% (n = 2) from the Adamaoua region.

Of the 2207 records, 2198 [99.6% (95% CI, 99.2%–99.8%)] were of HIV-1 group M, the majority being the *CRF02_AG* (62.7%), A_1 (8.2%), G (5.7%), and F_2 (3.9%). Nine HIV-1 group O records were identified: 0.4% (95% CI, 0.2%–0.8%); whereas no group N nor group P records were present (Supplemental Figure S1). Of the nine individuals harboring these group O strains, the mean age was 43 ± 12 years, five were males (55.6%), median (IQR) duration since HIV diagnosis was 627 (423 to 775) weeks, median (IQR) viral load was 12,385 (5340 to 72,682) copies/mL, and median (IQR) CD4 count was 52 (39 to 228) cells/mm^3. A detailed description of the nine individuals with group O strains is presented in Table 1.

Performance of Rapid Subtyping Tool Assignation of Group O Strains

The performance of commonly used rapid subtyping tools in the laboratory's clinical routine was evaluated for the detection of group M strains (Table 2). Only eight of the nine samples were used for this analysis (one was assigned group O by serotyping, with unsuccessful amplification after several attempts). Stanford HIVdb program classified all samples as unknown, whereas HIV REGA suggested potential recombinants for all strains, except for one sample, which was unassigned. HIV Comet classified all samples as HIV-1 group O with 100% support for all samples, as well Ge2pheno and HIV Basic Local Alignment Search Tool, which also classified all samples as group O, although their similarity support was lower.

Performance of Rapid Diagnostic Tests

Of the nine group O samples identified, eight had remnant plasma aliquots available for laboratory analyses with RDTs. Of the RDTs used, One step, KHB, and ACRO Rapid Test (HIV1/2 and p24) detected all eight samples (100% sensitivity), First Response HIV1-2.0 detected seven of eight (87.5%), whereas Determine HIV1/2 detected six of eight (75.0%). Of all samples that reacted to ACRO Rapid Test (HIV1/2 and p24), reactivity was seen only for HIV-1, whereas no sample was reactive to HIV-2 (indicating absence of HIV-1 and HIV-2 co-infection), nor p24. Overall performance of the two-test algorithm (Determine HIV1/2 as first test) in group O detection would be 75% (6/

Table 2 Performance of Commonly Used Subtyping Tools in the Detection of HIV-1 Group O Strains

ID	Stanford HIVdb	COMET HIV-1	REGA HIV subtyping tool	Geno2pheno	HIV BLAST
GO-CAM-20	Unknown	O (100%)	F_1, potential recombinant (6%)	O (91%)	O (93%)
GO-CAM-62	Unknown	O (100%)	J, potential recombinant (77%)	O (94%)	O (96%)
GO-CAM-95	Unknown	O (100%)	A_1, potential recombinant (5%)	O (87%)	O (93%)
GO-CAM-75	Unknown	O (100%)	NA	O (89%)	O (90%)
GO-CAM-29	Unknown	O (100%)	A_1, potential recombinant (26%)	O (87%)	O (93%)
GO-CAM-94	Unknown	O (100%)	Recombinant (NA)	O (88%)	O (91%)
GO-CAM-02	Unknown	O (100%)	C, potential recombinant (35%)	O (87%)	O (91%)
GO-CAM-83	Unknown	O (100%)	J, potential recombinant (50%)	O (78%)	O (89%)

The subtyping analysis was performed on eight available sequences of the nine group O identified.
BLAST, Basic Local Alignment Tool; ID, identification; NA, not assigned.

8), whereas the performance of the three-test algorithm (First Response HIV1-2.0 as first test) would be 87.5% (7/8). Rapid test results are summarized in Table 3.

HIV Biomarker Characterization

Six samples were positive on biomarker characterization using INNO-LIA, whereas two were undetermined, showing just 1+ reactivity for only sgp120. These two samples were the same samples that did not react on Determine HIV1/2. Four samples reacted to p24, as opposed to zero with the p24 RDT (ACRO), suggesting lower p24 detection threshold by the RDT. The specific biomarkers identified in each sample are presented in Table 4.

Genotypic Profiles of HIV-1 Group O Strains

The mutational profiles of all eight individuals with available sequences are presented in Supplemental Table S1. In reverse transcriptase, seven of them (87.5%) harbored the Y181C mutation, whereas the individual without Y181C had the A98G and V179E. Seven of the eight individuals all had the A98G and V179E mutations occurring together, whereas one had just A98C. Regarding other mutations, six individuals harbored the V118C + L210Y mutations, one had V118C + L210D, and the last had only the L210Y mutation. In protease, the K20C mutation was found in five individuals, with other mutations like I13A, I62V, and I93L also frequent.

Discussion

In this current study, no group N or P was observed, whereas nine group O strains were identified (0.4%). Indeed, HIV-1 group O has been emerging in Cameroon since the 1980s,[27–29] with epidemiologic rates of approximately 1.1% in a large study conducted between 2006 and 2007,[24] and 0.6% in another study conducted between 2006 and 2013.[4] Molecular characterization of HIV-1 specimens between 2011 and 2015 from rural southern Cameroon also showed incredible diversity, including 7 subtypes, 12 CRFs, 6 unclassified, 24 group O infections, and 2 group N infections,[14] whereas Oliveira et al[30] also revealed 10 cases involving group O, with 4 dual group M and O infections, and six group M/O recombinants. These dynamics are in line with the data observed in this study, which show a rate of <1% among individuals received at CIRCB. Taking into

Table 3 Results of Rapid Diagnostic Tests on Samples With Group O Strains

Sample ID	First Response HIV1-2.0	One Step	Determine HIV1/2 set (Abbott)	KHB colloidal gold	ACRO Rapid Test (HIV1/2 and P24)
GO-CAM-20	R1	R	NR	R	R1; NR: p24
GO-CAM-62	R1	R	R	R	R1; NR: p24
GO-CAM-95	R1	R	NR	R	R1; NR: p24
GO-CAM-75	R1	R	R	R	R1; NR: p24
GO-CAM-020	NR	R	R	R	R1; NR: p24
GO-CAM-29	R1	R	R	R	R1; NR: p24
GO-CAM-94	R1	R	R	R	R1; NR: p24
GO-CAM-02	R1	R	R	R	R1; NR: p24
Total (N = 8)*	7/8	8/8	6/8	8/8	8/8
% (95% CI)	87.5 (52.9–99.3)	100 (67.6–100)	75 (40.9–92.9)	100 (67.6–100)	100 (67.6–100)

*This analysis was performed on the eight available remnant plasma aliquots.
ID, identification; NR, non-reactive; R, reactive; R1, reactive to HIV-1.

Table 4 Molecular Characterization of Reactive Biomarkers in the Eight Samples with HIV-1 Group O

Sample ID	sgp120	gp41	p31	p24	p17	sgp105	gp36	Result
GO-CAM-20	1+	/	/	/	/	/	/	**UND**
GO-CAM-62	1+	1+	2+	2+	2+	/	/	POS
GO-CAM-95	1+	/	/	/	/	/	/	**UND**
GO-CAM-75	2+	2+	1+	/	/	/	/	POS
GO-CAM-020	1+	2+	2+	2+	2+	/	/	POS
GO-CAM-29	1+	1+	1+	2+	/	/	/	POS
GO-CAM-94	3+	3+	3+	3+	3+	/	/	POS
GO-CAM-02	1+	2+	2+	/	/	/	/	POS

The table shows shows the antigens detected by the INNO-LIA assay in the analyzed samples. Evaluated antigens for HIV-1 are the envelope proteins (sgp120 and gp41), gag proteins (p17 and p24), and pol (p31); and for HIV-2 are envelope proteins (sgp105 and gp36). Undetermined results are boldfaced.

ID, identification; POS, positive; UND, undetermined.

consideration the previous HIV-1 group O prevalences, there is a visible decreasing prevalence of group O (Supplemental Figure S2).[4,24,25] Meanwhile, the convenience sampling, and long duration of infection in these individuals (>10 years in average, the most recent in 2016), hampers the timing of the decay of group O because most probably these infections are relatively old, and possibly not clear evidence about the situation of today's circulation of group O. However, the absence of any new strains recently supports that the prevalence of these group O strains has remarkably decreased. Furthermore, the concomitant decrease in overall HIV prevalence in Cameroon (favored by improved HIV-1 services, universal test and treat measures, as well as a decrease in risky sexual behaviors),[31] the scale-up of more potent antiretroviral drugs, such as boosted protease inhibitors and integrase strand transfer inhibitors, more active on group O strains compared with the NNRTI (*https://www.who.int/news/item/16-07-2021-who-publishes-new-consolidated-hiv-guidelines-for-prevention-treatment-service-delivery-monitoring*, last accessed July 19, 2025), would have also contributed to the decrease in HIV-1 group O. Last, the suggested lower fitness of HIV-1 group O compared with group M strains also contributes to its low prevalence and limited geographic spread.[32] These aforementioned points contribute to a slow spread of these strains,[33] leaving the opportunity for eradication in coming years. Interestingly, it is known that HIV would have originated from zoonotic crossover,[34] and some evidence shows that most group O strains responsible for epidemiologic spread in West and Central Africa originated by cross-species transmission from western lowland gorillas.[35] In an era where human-to-human group O prevalence is expectedly declining because of decreased human-to-human transmission, it may also be necessary to explore zoonotic transmission, toward definite eradication.

Varying results were observed in the detection of group O samples by RDTs used. Three of the RDTs [One Step, KHB, and ACRO Rapid Test (HIV1/2 and p24)] accurately tested all samples (100%), whereas First response succeeded in seven of eight samples (87.5%) and Determine HIV1/2 in six of eight samples (75%). Diagnostic inaccuracies have been reported in the past on samples with HIV-1 group O.[10,36] A 2007 study of 10 HIV-1 group O samples in Cameroon showed 100% [10/10 detection with Determine HIV1/2, ImmunoComb II, and SD Bioline, compared with 80% (8/10) with HIV(1+2) Strip and 20% (2/10) with retro-check].[10] The decrease in detection of HIV-1 group O by the Determine test (75%, 6/8) during the present evaluation would appear to be due to the high genetic variability of this viral strain over time and supported by the difference of >10 years between the two studies. In one case, the escape of HIV-1 group O from serologic tests was due to mutations in the immunodominant epitopes of gp41 in a Cameroonian woman.[12] Similar variations in antigenic epitopes could therefore explain the poor detection of HIV-1 group O in the current era. Indeed, the two unreactive samples on Determine also had inconclusive results with INNO-LIA as reactivity (1+) was found only for sgp120 (no reactivity for all other markers, including gp41). Although previous data have shown reduced sensitivity of serologic assays with chronic antiretroviral therapy, the suggested explanation was a progressive loss of seropositivity status following prolonged viral undetectability.[37] However, given the high viral loads in these two individuals (Table 1) with unreactive and undetermined results on Determine and INNO-LIA, respectively, it is less likely that this was due to low levels of antibody/antigen. The performance of determine would reduce the performance of the two-test algorithm (Determine HIV1/2 as first rest) in the detection of HIV-1 group O. The detection of 87.5% (7/8) of HIV-1 group O strains by First Response would indicate that the test can react to most HIV-1 group O strains currently circulating in Cameroon. These observations are in line with the plausible use of the present three-test algorithm in Cameroon (First Response as first test), which could be more sensitive in identifying HIV group O strains compared with the previous two-test algorithm. Indeed, under the supervision of the Minister of Public Health in Cameroon, the current evaluation was performed as an additional component in the selection/evaluation process of tests to be used in the three-test

algorithm, from a series of eight WHO prequalified tests. According to the results of the full-scale evaluation, tests were prioritized following specific criteria, including a low risk of false reactivity (<2%), absence of invalid results (to minimize waste of resources), tests from different manufactures (to ensure varying test principles), cost-effectiveness, and the sensitivity in the detection of HIV-1 group O evaluated on the best performing tests. The currently chosen tests in the three-test algorithm performed best, motivating their preference in selection. Furthermore, of the three tests selected in this three-test algorithm, two of three detected all group-O cases, whereas just one (First Response) missed one case. However, a previous evaluation on group O samples from 1997 to 1998 showed 100% performance of First Response in detecting group O (Supplemental Table S2). Also, among the antigens on the First Response nitrocellulose membrane is a gp41 including group O, which should make it sensitive to group O. The slightly lower performance of First Response observed in this current analysis could therefore be linked to increased genetic evolution in the current samples compared with older strains in previous analyses, differences in antibody titers, or a potential lot-to-lot variation, which was not ruled out in the current study. Nonetheless, these results therefore are clinically relevant in a real-life setting where these variations may occur. The current observations, coupled with previous studies[4,9,10] on the dynamics of HIV-1 in Cameroon and its challenges in routine diagnostics, reveal the need to periodically update diagnostic tests according to the evolution of the virus over time. Despite the small number of samples used in this evaluation, the declining HIV-1 group O prevalence confirms the increasing scarcity in the number of available group O samples for such analyses. Also, previous evaluations have also been performed in small numbers of samples, even in the validation of diagnostic tests. Furthermore, considering the last prevalence before this study of 0.6% in Cameroon, the current analysis is therefore sufficient to provide at least good confidence in the observed findings. Nonetheless, in the ongoing implementation phase of the three-test algorithm in collaboration with the National AIDS Control Committee and the HIV reference laboratory (CIRCB), any identified group O cases would be subsequently analyzed.

As concerns rapid subtyping tools, which are useful in routine clinical practice, variable sensitivities were observed toward classifying these group-O strains, with HIV COMET appearing the most sensitive. Although performance might have been affected by the fact that these are just partial sequences from Sanger sequencing, this observation encourages periodical updates of these platforms, for more accurate detection of these strains and better interpretation of virological results. Most individuals harbored common major NNRTI mutations, described among HIV-1 group O strains (such as A98G, V179E, and Y181C), as well as other accessory mutations in the reverse transcriptase region. Five of the eight individuals in this study initiated therapy with first-generation NNRTI-based regimens (efavirenz or nevirapine), inevitably leading to subsequent failure and the observed advanced resistance profiles at the time of genotyping. The V179E and A98G mutations were observed in seven of the eight individuals. Together with the Y181C mutation (which mostly affects the first-generation NNRTI), these mutations when present together would considerately hamper the potential efficacies (according to the Stanford algorithm scores) of even the second-generation NNRTI (to a lesser extent doravirine), jeopardizing thus the potential use of these promising regimens. Nonetheless, adequate treatment adjustments were proposed (Supplemental Table S2) with respect to observed resistance profiles. Although given the cross-sectional nature of this study, it is difficult to ascertain the evolution on these optimized regimens in terms of current viral loads or genotypic profiles, previous findings have shown high rates of viral suppression following genotypic resistance test guided switches,[38] with a high likelihood of good virologic control in these individuals following current transition to dolutegravir-based regimens. In the protease, minor mutations, such as I13A, K20C, I62V, and I93L, were present in most individuals. Previous studies have revealed polymorphisms in approximately 34% of the protease gene in group O sequences, although no naturally occurring primary resistance mutations have been described to date.[39] Although most interpretation algorithms suggest no impact on protease inhibitors, the phenotypic impact of these polymorphisms has not yet been fully studied.[40] This problem of natural resistance of group O strains (definitely to first-generation NNRTI and possibly to NRTI) could pose significant treatment challenges in settings still using NNRTI-based regimens in initial management of PWH.[13] To add, in Cameroon, pretreatment drug resistance (HIV-1 group M) is above the 10% threshold.[41] The use of NNRTI-based therapies will imply functional bitherapies, which could rapidly increase risks of resistance to other molecules, potentially jeopardizing subsequent regimens. These findings further support the WHO's plan for roll-out in dolutegravir-based regimens (fully active on group O), and progressive phase out of NNRTI-based regimens, in this context, which is endemic to HIV-1 group O, with also high rates of NNRTI pretreatment drug resistance even in group M strains.[41,42]

Although ensuring accurate testing is essential to prevent misdiagnosis, the costs and economic implications of testing strategies are important to consider. The two- and three-test algorithms are both serial algorithms, meaning consecutive tests are needed to provide an HIV-positive diagnosis and not all the tests are performed at once. Therefore, testing cost is primarily driven by the volume of clients who receive the first test (A1) in the algorithm (Preventing HIV Misdiagnosis: Implementation Guide, *https://www.who.int/publications/i/item/9789240092136#:~:text=The%20WHO-recommended%20HIV%20testing%20strategy%2C%20along%20with%20quality,misdiagno*

sis%20and%20unnecessary%20initiation%20of%20costly%20lifelong%20treatment, last accessed October 3, 2025). For this reason, shifting from the two- to three-test algorithm should have little impact in the overall testing cost, confirmed by modeling studies, which suggest only approximately 2.5% difference in cost between both strategies at a 5% positivity.[43] On a more practical note, considering the Cameroonian context and the currently evaluated tests, a simple cost evaluation can be performed using the latest rapid diagnostic test prices as per Global Fund report on pricing (*https://www.theglobalfund.org/media/2ffcrcra/psm_hivrdtreferencepricing_table_en.pdf*, last accessed October 3, 2025). According to this report, the first tests (A1) for both algorithms in the current study (First Response and Determine for the three- and two-test algorithms, respectively) are in the same price range ($0.71 to $0.90), whereas all the other tests, A2 (for the three- and two-test algorithm) and A3 (for the three-test algorithm), are all in the same price range ($0.51 to $0.70). Hence, estimating the cost of identifying positive cases on a sample of 100,000 individuals in this Cameroonian context with 2.7% national prevalence (assuming similar performance of both A1 tests in negative predictive value), 100,000 individuals would benefit from A1, 5000 for A2 (two- and three-test algorithms), and 2500 for A3 only in the three-test algorithm (estimating that 50% on A2 would remain positive). Considering an average cost of $0.81 for both A1 tests and $0.61 for the other tests, the estimated cost of testing 100,000 individuals in this context will be $83,470.50 for the three-test algorithm versus $82,647.00 for the two-test algorithm. This means just a 1% cost increment in using the three-test algorithm compared with the two-test algorithm (a negligeable difference). Adding the cost associated with misdiagnosis with the two-test algorithm (2% higher risks of false positives in the two-test algorithm), this becomes extremely high, as it includes unnecessary laboratory testing for response to treatment, lifelong treatment costs (or further costs of retesting to resolve misdiagnosis), as well as personal financial and social costs.[44] Regarding efficiency (cost/effectiveness ratio) in detecting group O, better efficiency was obtained with this three-test algorithm ($954.00) compared with the two-test algorithm ($1102.00). Overall, these elements highlight superiority in terms of diagnostic benefits of the three-test algorithm when considering the impacts based on efficiency.

Conclusion

In this West/Central African country, HIV-1 group N and P are scarce. In contrast, group O remains low (<1%), with a potential declining trend, indicating a gradual decline owing to the effectiveness of more potent regimens over the years as countries move toward viral elimination. On eight HIV-1 group O strains, the two-test algorithm detected 75% against 87.5% for the three-test algorithm. Thus, transitioning to the defined three-test algorithm could lead to a slightly improved diagnostic performance on currently circulating HIV-1 group O. However, updating RDTs following viral dynamics is crucial, to prevent misdiagnoses that consequently limit the global efforts toward HIV elimination. In this era of potent dolutegravir-based regimens, identification of all group O strains, especially in this endemic setting, is of paramount importance for their control and the AIDS elimination as a global threat.

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Author Contributions

J.F. conceptualized the study; J.F., C.A.C., and D.A.T.K. developed methods; J.F., M.M.S., and F.C.-S. validated data; O.E.G.B.A., D.A.T.K, S.C.D.J., E.N.J.S., A.D.N., S.M.S., R.K., N.N.F., and M.C.T.T. analyzed and interpreted data; O.E.G.B.A., S.C.D.J., V.K.M., A.C.K., R.A.N.M., A.M.K.N., D.T.A.N., and D.A.T.K. performed experiments; J.F., A.N., and I.O.B.E.O. obtained resources; V.K.M., N.-K.E., and A.C.K. curated data; E.N.J.S., A.D.N., and C.A.C. wrote the manuscript; J.F., M.M.S., C.F., J.B.P., A.K., L.M., S.C.B., E.S.T., J.d.D.A., R.O., P.O., G.A.E.B., O.E.G.B.A., N.M., H.H., A.-E.N.-N., A.-C.Z.-K.B., Y.B., A.J.N., C.N.N., J.N.T., E.E.M., F.C.-S., G.C., C.K., I.O.B.E.O., E.T., and R.A.A. reviewed and edited the manuscript; N.N., V.C., C.-F.P., A.N., and M.M. supervised the study; and all authors have read and agreed to the published version of the manuscript.

Disclosure Statement

None declared.

Supplemental Data

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