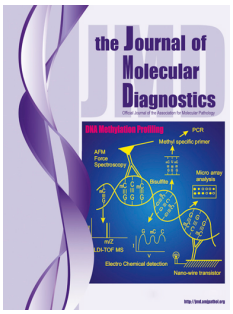
Journal Pre-proof



Updates on the clinical epidemiology of HIV-1 group O strains in Cameroon and potential implications on diagnosis and treatment strategies

Joseph Fokam, Collins Ambe Chenwi, Désiré Augustin Takou Komego, Odile Estelle Grâce Beloumou Angong, Sandrine Claire Djupsa Njdeyep, Ezechiel Ngoufack Jagni Semengue, Alex Durand Nka, Aude Christelle Ka'e, Vincent Kamaël Mekel, Aurelie Minelle Kengni Ngueko, Naomi-Karel Etame, Rachel Audrey Nayang Mundo, Samuel Martin Sosso, Rachel Kamgaing, Nadine Fainguem, Derrick Tambe Ayuk, Charles Fokunang, Michel Carlos Tchouaket Tommo, Joelle Bouba Pamen, Alice Ketchaji, Lydie Mvondo, Serge Clotaire Billong, Edith Saounde Temgoua, Jean de Dieu Anoubissi, Roger Onana, Pierrette Omgba, Georges Alain Etoundi Balla, Nadia Mandeng, Hadja Hamsatou, Anne-Esther Njom-Nlend, Anne-Cecile Z-K Bissek, Yagai Bouba, Aubin Joseph Nanfack, Celine Nguefeu Nkenfou, Judith N. Torimiro, Emmanuel Eben Moussi, Maria Mercedes Santoro, Francesca Cecchereni-Silberstein, Giulia Cappelli, Charles Kouanfack, Isidore Oscar Bienvenu Etogo Ondigui, Elvis Temfack, Nicaise Ndembi, Vittorio Colizzi, Carlo-Federico Perno, Rogers Awoh Ajeh, Alexis Ndjolo, Malachie Manaouda

PII: S1525-1578(25)00279-X

DOI: https://doi.org/10.1016/j.jmoldx.2025.10.008

Reference: JMDI 1601

To appear in: *The Journal of Molecular Diagnostics*

Received Date: 22 April 2025

Revised Date: 3 October 2025

Accepted Date: 22 October 2025

Please cite this article as: Fokam J, Chenwi CA, Takou Komego DA, Grâce Beloumou Angong OE, Djupsa Njdeyep SC, Jagni Semengue EN, Nka AD, Ka'e AC, Mekel VK, Kengni Ngueko AM, Etame N-K, Nayang Mundo RA, Sosso SM, Kamgaing R, Fainguem N, Ayuk DT, Fokunang C, Tchouaket Tommo MC, Pamen JB, Ketchaji A, Mvondo L, Billong SC, Temgoua ES, de Dieu Anoubissi J, Onana R, Omgba P, Etoundi Balla GA, Mandeng N, Hamsatou H, Njom-Nlend A-E, Z-K Bissek A-C, Bouba Y, Nanfack AJ, Nkenfou CN, Torimiro JN, Moussi EE, Santoro MM, Cecchereni-Silberstein F, Cappelli G, Kouanfack C, Bienvenu Etogo Ondigui IO, Temfack E, Ndembi N, Colizzi V, Perno C-F, Ajeh RA, Ndjolo A, Manaouda M, Updates on the clinical epidemiology of HIV-1 group O strains in Cameroon

and potential implications on diagnosis and treatment strategies, *The Journal of Molecular Diagnostics* (2025), doi: https://doi.org/10.1016/j.jmoldx.2025.10.008.

Updates on the clinical epidemiology of HIV-1 group O strains in Cameroon and potential implications on diagnosis and treatment strategies

Short Title: Diagnosis of HIV-1 group O in Cameroon

Joseph Fokam[1,2,3,4,5], Collins Ambe Chenwi[1,4,6,£], Désiré Augustin Takou Komego[1,4,£], Odile Estelle Grâce Beloumou Angong[1,6,£], Sandrine Claire Djupsa Njdeyep[1], Ezechiel Ngoufack Jagni Semengue[1,4], Alex Durand Nka[1], Aude Christelle Ka'e[1] , Vincent Kamaël Mekel[1,6], Aurelie Minelle Kengni Ngueko[1,5], Naomi-Karel Etame[1,5], Rachel Audrey Nayang Mundo[1], Samuel Martin Sosso[1], Rachel Kamgaing[1], Nadine Fainguem[1], Derrick Tambe Ayuk[1,5], Charles Fokunang[5], Michel Carlos Tchouaket Tommo[1], Joelle Bouba Pamen[5,7,8], Alice Ketchaji[8], Lydie Mvondo[8], Serge Clotaire Billong[5,8], Edith Saounde Temgoua[2,9], Jean de Dieu Anoubissi[2], Roger Onana[2], Pierrette Omgba[2,8], Georges Alain Etoundi Balla[8], Nadia Mandeng[8], Hadja Hamsatou[10], Anne-Esther Njom-Nlend[3], Anne-Cecile Z-K Bissek[5,11], Yagai Bouba[1,12], Aubin Joseph Nanfack[1], Celine Nguefeu Nkenfou[1], Judith N. Torimiro[1,6], Emmanuel Eben Moussi[1], Maria Mercedes Santoro[6], Francesca Cecchereni-Silberstein[6], Giulia Cappelli[1,13,14], Charles Kouanfack[4,15], Isidore Oscar Bienvenu Etogo Ondigui[16], Elvis Temfack[17], Nicaise Ndembi[17,18], Vittorio Colizzi[1,19,20], Carlo-Federico Perno[1,21], Rogers Awoh Ajeh[2,22,*], Alexis Ndjolo[1,5,*], Malachie Manaouda[23,*]

1. *Chantal BIYA International Reference Centre for HIV/AIDS prevention and management, Yaoundé, Cameroon;*
2. *Central Technical Group, National AIDS Control Committee, Ministry of Public Health, Yaoundé, Cameroon;*
3. *Faculty of Health Sciences, University of Buea, Buea, Cameroon;*
4. *National HIV drug resistance working group, Yaoundé, Cameroon;*
5. *Faculty of Medicine and biomedical Sciences, University of Yaoundé I, Yaoundé, Cameroon;*
6. *Faculty of Medicine and surgery, University of Rome Tor Vergata, Rome, Italy;*
7. *Department of Heritage and Financial Resources, Ministry of Public Health, Yaoundé, Cameroon;*
8. *Department of Disease, Epidemics and Pandemic Control, Ministry of Public Health, Yaoundé, Cameroon;*
9. *Management Sciences for Health, Yaoundé, Cameroon;*
10. *National Public Health Laboratory, Ministry of Public Health, Yaoundé, Cameroon;*

11. *Division of Health Operational Research, Ministry of Public Health, Yaoundé, Cameroon;*
12. *UniCamillus - Saint Camillus International University of Health Sciences, Rome, Italy*
13. *Institute of Biological Systems, National Research Council, Rome, Italy;*
14. *Laboratory for Major Tropical Epidemics, University Hospital Complex, N'djamena, Chad;*
15. *Faculty of Medicine and Pharmaceutical Sciences of the University of Dschang, HIV Treatment Centre of the Yaoundé Central Hospital, Centre for research on Emerging and Re-emerging Diseases, Yaoundé, Cameroon;*
16. *World Health Organisation, Country Office, Yaoundé, Cameroon;*
17. *Africa Centres for Disease Control and Prevention, Addis Ababa, Ethiopia;*
18. *Institute of Human Virology, University of Maryland School of Medicine, MD, USA;*
19. *EUROBIOPARK and UNESCO Board of Biotechnology, University of Rome Tor Vergata, Rome, Italy;*
20. *Faculty of Medicine, CHU Bon-Samaritain, N'djamena, Chad;*
21. *Bambino Gesù Pediatric Hospital, IRCCS, Rome, Italy;*
22. *Global Funds and Partners Subvention Coordination Unit, Ministry of Public Health, Yaoundé, Cameroon;*
23. *Ministry of Public Health, Yaoundé, Cameroon.*

Corresponding Author:
Joseph Fokam
Chantal BIYA International Reference Centre for research on HIV/AIDS prevention and management (CIRCB), PO Box 3077, Yaoundé, Cameroon; Central Technical Group, National AIDS Control Committee, Ministry of Public Health, Yaoundé, Cameroon; National HIV drug resistance working group, Yaoundé, Cameroon; TEL: +237 676927690 Emails: joseph.fokam@m365.cnls.cm and josephfokam@gmail.com.

£: contributed equally as second authors; *: contributed equally as senior authors

Declaration of conflicting interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication.

Funding statement

This work was funded by the CIRCB, under the 2023/2024 annual budget plan. The World Health Organization provided support for laboratory reagents and consumables used during the verification study of the 3-test algorithm.

ABSTRACT

Cameroon is an epicentre of diverse HIV-1 strains, with challenges in diagnosis and disease management. The objective herein was to update the prevalence of HIV-1 non-M and compare diagnostic-performance of the 2-test vs. 3-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. Molecular-phylogeny and rapid-subtyping were performed for identifying HIV-1 non-M. Performances of rapid diagnostic tests (RDTs) used in the 2-test (determine and KHB) vs. 3-test (First Response, One Step and KHB) algorithms were evaluated on non-M, with ACRO Rapid Test (HIV1/2&p24) as independent RDT. No group-N (0%) nor P (0%) were found while 09 group-O strains were identified (0.4%; 95%CI: 0.2%-0.8%). For individuals harbouring group-O, (mean-age, 43±12 years; 50% female) median [IQR] duration since HIV-diagnosis was 627 [423-775] weeks, median [IQR] viremia, 12 385 [5 340-72 682] copies/ml and median [IQR] CD4 count [IQR], 52 [39-228] cells/mm^3. One Step, KHB and ACRO Rapid Test (HIV1/2&p24) detected 8/8 group-O (100%-performance), First Response HIV1-2.0, 7/8 (87.5%-performance) and Determine HIV1/2, 6/8 (75%-performance), p=1.00. In this Cameroonian setting, HIV-1 group-N and P are scarce while group-O remains low (<1%). Transitioning from the 2-test (75%-performance) to the 3-test algorithm (87.5%-performance) could lead to improved diagnostic performance on currently circulating HIV-1 group-O, calling for updates in RDTs to adapt to viral dynamics.

INTRODUCTION

With over 40 million people living with HIV (PWH) worldwide, HIV/AIDS remains a global public health priority especially in sub-Saharan Africa (SSA) which harbours about two-thirds of the global burden. West and Central Africa (WCA) 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, 19/07/2025). With scale-up of antiretroviral therapy (ART) and improved therapeutic strategies, significant progress has been made towards attaining the Joint United Nations Programme on HIV/AIDS (UNAIDS) 95-95-95 objectives. In 2024, 87% of PWH knew their status, 89% of whom were on ART and 94% of those treated were virally suppressed (https://www.unaids.org/en/resources/fact-sheet). Though encouraging, the most significant gap remains within the first 95 concerning diagnosis. Indeed, the last two indicators depend on the first, and therefore efforts towards achieving this should be given major priority[1]. In WCA, 80% of PWH knew their status 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 19/07/2025), necessitating thus 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, 19/07/2025). The use of highly sensitive and specific tests (either RDT or enzyme immunoassays), prequalified by WHO is crucial, for accurate classification of tested individuals.

As the epicentre of HIV/AIDS, the west/central African region (and Cameroon in particular) has high HIV genetic diversity (harbouring all HIV types, groups and subtypes)[2–6], which could influence diagnostic accuracy and possibility 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*., in 2009 showed that about 80% of PWH with *group O* do not receive ART due to inaccuracies in diagnosis linked to low sensitivities of rapid diagnostic tests (RDT)[10]. 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 non-nucleoside reverse-transcriptase inhibitors (NNRTI) which were the mainstay of first-line 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 (WHO publishes new Consolidated HIV guidelines for prevention, treatment, service delivery & monitoring. https://www.who.int/news/item/16-07-2021-who-publishes-new-consolidated-hiv-guidelines-for-prevention-treatment-service-delivery-monitoring; last accessed, 19/07/2025). Although some evidence exists on the circulation of *non-group M* strains exists, 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 2-test to 3-test diagnostic algorithm as recommended by the WHO (2.7% national prevalence), the evaluation of the new 3-test algorithm is crucial to ensure accurate and timely detection of all circulating HIV strains and improve our 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 till 2023, therefore contributing updated information on the clinical epidemiology of these non-M strains. More importantly, this study evaluates tests used in the previous 2-test algorithm, as compared to the incoming 3-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 2-test vs. 3-test algorithm on the detection of circulating HIV-1 *non-group-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, Cameroon, on clinical samples referred for genotypic resistance testing at CIRCB and stored in the CIRCB-Biobank between 2006 up till 2023. The CIRCB is a WHO HIVResNet (HIV Drug Resistance Network) national laboratory and is a large-scale Reference Centre 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 (CARE) electronic database, we searched for all records with HIV-1 non-M strains, up till December 2023. These consisted of records of individuals previously diagnosed and followed up at clinical centres (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 harbouring non-*group M* strains were selected, and their samples retrieved from the CIRCB-Biobank for serological testing. Each sample had at least two cryotubes (1ml 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 CARE database for molecular phylogeny to confirm of viral strains (sequences available at: *https://www.ncbi.nlm.nih.gov/nucleotide/*, accession numbers PQ893190, PQ893192, PQ893193, PQ893194, PQ893195, PQ893196, PQ893197, and PQ893198, last accessed 19/07/2025). Reference sequences of pure subtypes, circulating recombinant forms (CRFs) and non-M groups in West and Central 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 performed using maximum likelihood method on IQ-TREE version 2.0.7[17], and tree visualization and editing performed using Figtree version 1.4.4 (available at http://tree.bio.ed.ac.uk/software/figtree/ ; last access date 19/07/2025). The classification of non-*group 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 lastly Basic Local Alignment Search Tool (BLAST) (https://www.hiv.lanl.gov/content/sequence/BASIC_BLAST/basic_blast.html; last access date 19/07/2025). Genotypic profiles of all group O sequences were also analysed using Stanford HIVdb version 9.4.1.

Serological testing on HIV-1 non-*group M*

Rapid diagnostic tests were carried out following manufacturer's instructions, using WHO prequalified used in the 2-test[23] and 3-test algorithm (Ministerial decision: N° 0098/D/MINSANTE, accessible at https://cnls.cm/catdocument/rapports-annuels, on 23 October 2025). The 2-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 manufacturers; 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 also HIV-1 *group O* as specified by the manufacturers. The tests in the 3-test algorithm are; first test: First Response HIV1-2.0 Card Test (Premier Medical Corporation Private Limited; Gujarat, India); second test: One Step Anti-HIV (1&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; Shangai, China) all also sensitive in detecting HIV-1, HIV-2 and HIV-1 *group O* as specified by manufacturers. An additional internal test was added during laboratory analysis, to increase sensitivity especially in detecting early infections (ACRO Rapid Test HIV1/2 & p24 (Acro Biotech Inc; Montclair, USA). The reactive biomarkers were characterized using INNO-LIA HIV I/II Score (Fujirebio Europe N.V;

Gent, Belgium) as per the manufacturer's instructions. All RDTs were carried out by trained technicians at CIRCB, repeated in pairs for each test, and results reported by 6 individuals independently, with the consensus used as the result. The results of INNO-LIA were interpreted by a team composing of the laboratory head and 3 senior laboratory scientists.

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 non-*group 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 analyses

Epi info version 7.2.6 statistical software standalone version (available at https://www.cdc.gov/epiinfo; last access date 19/07/2025) was used for statistical analysis. The mean, median, standard deviation (SD) and inter-quartile range (IQR) were used for non-nominal variables. The Student T-test and Chi-square/Fisher exact test were used to compare continuous and categorical variables, respectively (where necessary for the entire population) with p-values of ≤ 0.05 considered statistically significant. Using prevalences of HIV-1 group O over the years as obtained from previous studies, a trend graph was prepared using GraphPad prism, version 8.0.1.

Ethical Considerations

The study was conducted in accordance with the Helsinki Declaration[24]. Samples used from the CIRCB biobank had previously 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 analysed from the CARE database, median age [IQR], 38 [19-47] years, 67.8% (n=1 496) 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, 12.9% (n=285) from the North West, 9.6% (n=212) from the West, 6.4% (n=140) from the South West, 4.7% (n=103) from the East, 2.6% (n=57) from the North, 2.2% (n=49) from the extreme North,1.8% (n=39) from the south 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* were identified: 0.4% (95%CI: 0.2%-0.8%), while no group N nor *group P* were present (Supplemental Figure S1). Of the nine individuals harbouring these *group O* strains, the mean age was 43±12 years, five were males (55.6%), median duration since HIV diagnosis was 627 [423-775] weeks, median viral load [IQR] was 12,385 [5340-72,682] copies/ml and median CD4 count [IQR] was 52 [39-228] cells/mm^3. A detailed description of the nine individuals with *group O* strains is presented in Table 1.

Performance of rapid subtyping tools assignation of *group O* strains

The performance of commonly used rapid subtyping tools in the laboratory's clinical routine were evaluated for the detection of *group M* strains (presented in Table 2). Only eight out of the 9 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' while 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 BLAST which also classified all samples as *group O* although their similarity support was lower.

226

227 **Performance of rapid diagnostic tests**

228 Of the 9 *group O* samples identified, eight had remnant plasma aliquots available for laboratory analyses
229 with RDTs. Of the RDTs used, One step, KHB and ACRO Rapid Test (HIV1/2 & p24) detected all 8
230 samples (100% sensitivity), First response HIV1-2.0 detected 7/8 (87.5%) while Determine HIV1/2
231 detected 6/8 (75.0%). Of all samples which reacted to ACRO Rapid Test (HIV1/2 & p24), reactivity was
232 seen only for HIV-1, while no sample was reactive to HIV-2 (indicating absence of HIV-1&2 co-infection),
233 nor p24. Overall performance of the 2-test algorithm (Determine HIV1/2 as first-test) in *group O* detection
234 would be 75% (6/8), while the performance of the 3-test algorithm (First Response HIV1-2.0 as first-test)
235 would be 87.5% (7/8). Rapid test results are summarized in Table 3.

236 **HIV biomarker characterization**

237 Six samples were positive on biomarker characterization using INOLIA, while two were undetermined,
238 showing just 1+ reactivity for only sgp120. These two samples were the same samples which did not
239 react on determine HIV1/2 (Abott). Four samples reacted to p24, as opposed to zero of with the p24
240 RDT (ACRO), suggesting lower p24 detection threshold by the RDT. The specific biomarkers identified
241 in each sample are presented in Table 4.

242 **Genotypic profiles of HIV-1 *group O* strains**

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

DISCUSSION

In this current study, no *group N* nor *P* were observed, while nine *group O* strains were identified (0.4%). Indeed HIV-1 *group O* has been emerging in Cameroon since the 1980s[25–27], with epidemiological rates around 1.1% in a large study conducted between 2006-2007[28], and 0.6% in another study conducted between 2006-2013[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* and 2 Group N infections[14], while Oliveira et al also revealed 10 cases involving *group O* with four dual *groups M* and *O* infections, and six groups M/O recombinants[29]. These dynamics are in line with the data observed in this study, which show a rate of less than 1% among individuals received at CIRCB. Taking into consideration the previous HIV-1 *group O* prevalences, there is a visible decreasing prevalence of *group O* (Supplemental Figure S2)[4,28,30]. Meanwhile, the convenience sampling, and long duration of infection in these individuals (more than 10 years in average, the most recent in 2016), hampers the timing of the decay of group-O since 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 (favoured by improved HIV-1 services, universal test and treat measures as well as a decrease in risky sexual behaviours) [31], the scale-up of more potent antiretroviral drugs such as protease inhibitors (PI/r) and integrase strand transfer inhibitors (INSTIs), more active on *group O* strains as compared to the NNRTI (https://www.who.int/news/item/16-07-2021-who-publishes-new-consolidated-hiv-guidelines-for-prevention-treatment-service-delivery-monitoring), would have also contributed to the decrease in HIV-1 *group O*. Lastly, the suggested lower fitness of HIV-1 *group O* as compared to *group M* strains also contributes to its low prevalence and limited geographical 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 epidemiological spread in WCA originated by cross-species transmission from western lowland gorillas[35]. In an era where human-to-human *group O* prevalence is expectedly declining due to decreased human-to-human transmission, it may also be necessary to explore zoonotic transmission, towards 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 &p24)) accurately tested all samples (100%), while First response succeeded in 7/8 samples (87.5%) and Determine HIV1/2 in 6/8 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 to 80% (8/10) with HIV(1+2) Strip and 20% (2/10) with retro-check[10]. The drop in detection of HIV-1 *groups 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 also by the difference of more than 10 years between the two studies. In one case, the escape of HIV-1 *group O* from serological 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 INNOLIA as reactivity (1+) was found only for sgp120 (no reactivity for all other markers including gp41). Though previous data has shown reduced sensitivity of serological 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 INNOLIA 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 2-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 3-test algorithm in Cameroon (First response as first test), which could be more sensitive in identifying HIV-*group O* strains as compared to the previous 2-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 3-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 3-test algorithm performed best, motivating their preference in selection. Furthermore, it is worth noting that of the three tests selected in this 3-test algorithm, 2/3 detected all group-O cases while just one (First Response) missed one case. However, a previous evaluation on *group O* samples from 1997-1998 showed 100% performance of First response in detecting *group O* (Supplemental Table S2). Also, it should be noted that 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 as compared to older strains in previous analyses, differences in antibody tires, 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 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 carried out in very 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 3-test algorithm in collaboration with the National AIDS Control Committee and the HIV reference lab (CIRCB), any identified group O cases would be subsequently analysed.

As concerns rapid subtyping tools, which are very useful in routine clinical practice, variable sensitivities were observed towards 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 virological results. Most individuals harboured common major NNRTI mutations, described amongst HIV-1 *group O* strains (such as A98G, V179E and Y181C), as well as other accessory mutations in the reverse transcriptase region. It is worth noting that 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 out 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), jeopardising 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 virological control in these individuals following current transition to dolutegravir-based regimens. In the protease, minor mutations such as I13A, K20C, I62V, I93L were present in most individuals. Previous studies have revealed polymorphisms in about 34% of the protease gene in *group O* sequences, though no naturally

occurring primary resistance mutations have been described till date[39]. Although most interpretation algorithms suggest no impact on PI/r, the phenotypic impact of these polymorphisms have 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 PLHIV[13]. To add, it should be noted that in Cameroon, pre-treatment drug resistance (HIV-1 *group M*) is above the 10% threshold[41]. The use of NNRTI-based therapies will imply functional bi-therapies, which could rapidly increase risks of resistance to other molecules, potentially jeopardizing subsequent regimens. These further support the WHOs plan for roll-out in DTG-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 pre-treatment drug resistance even in *group M* strains[41,42].

Though ensuring accurate testing is essential to prevent misdiagnosis, the costs and economic implications of testing strategies are important to consider. Of note, the 2-test or 3-test algorithms are both serial algorithms, meaning consecutive tests are needed to provide an HIV-positive diagnosis and not all the tests carried out 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,misdiagnosis%20and%20unnecessary%20initiation%20of%20costly%20lifelong%20treatment; last accessed 03/10/2025). For this reason, shifting from the 2-test to 3-test should have very little impact in the overall testing cost, confirmed by modelling studies which suggest only about 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

03/10/2025). According to this report, the first test (A1) for both algorithms in the current study (First Response and Determine for the 3- and 2- test algorithms respectively) are in the same price range ($0.71 - $0.90), while all the other tests, A2 (for the 3-test and 2-test algorithm) and A3 (for the 3-test algorithm) are all in the same price range ($0.51 - $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 (2- and 3-test algorithms) and 2500 for A3 only in the 3-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 $83,470.5 for the 3-test algorithm versus $82,647.0 for the 2-test algorithm. This means just a 1% cost increment in using the 3-test algorithm as compared to the 2-test algorithm (a very negligeable difference). Adding the cost associated with misdiagnosis with the 2-test algorithm (2% higher risks of false positives in the 2-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 3-test algorithm ($9534.0) as compared to the 2-test algorithm ($ 1 101.0). Overall, these elements highlight superiority in terms of diagnostic benefits of the 3-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 2-test algorithm detected 75% against 87.5% for the 3-test algorithm. Thus, transitioning to the defined 3-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.

Acknowledgements

Acknowledgements to the staff, interns and administration of CIRCB for enabling us to carry out this work and to all individuals whose samples were used in this study. Immense thanks go to the Cameroon Minister of Public Health, who spearheaded this analysis to enable programmatic actions.

Author contributions

Conceptualization, J.F.; methodology, J.F., C.A.C., D.A.T.K.; validation, J.F., M.M.S., and F.C.-S.; formal analysis/interpretation, G.B., D.T., S.D., E.N.J.S., A.K.D., S.M.S., R.K, N.F., M.T., investigation, V.M., A.K., R.A.N.M., and D.T.A., resources, A.N.; data curation, V.M., and N-K.E., F.A.; writing—original draft preparation, E.N.J.S., A.K.D., C.A.C.; writing—review and editing, J.F., M.M.S., C.F., J.B.P., A.S.A.A., A.K., L.M., S.C.B., E.S.T., J.D.N., R.O., P.O., G.A.E.B., N.M., H.H., A.E.N-N., A-C.Z-K.B., B.Y., 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., R.A.A.; super-vision, N.N., V.C., C-F.P., A.N., M.M. All authors have read and agreed to the published version of the manuscript.

REFERENCES

1. van Schalkwyk C, Mahy M, Johnson LF, Imai-Eaton JW. Updated Data and Methods for the 2023 UNAIDS HIV Estimates. J Acquir Immune Defic Syndr, 2024, 95:e1–4
2. Yamaguchi J, Bodelle P, Vallari AS, Coffey R, McArthur CP, Schochetman G, Devare SG, Brennan CA. HIV Infections in Northwestern Cameroon: Identification of HIV Type 1 Group O and Dual HIV Type 1 Group M and Group O Infections. AIDS Research and Human Retroviruses, 2004, 20:944–57
3. Vallari A, Holzmayer V, Harris B, Yamaguchi J, Ngansop C, Makamche F, Mbanya D, Kaptué L, Ndembi N, Gürtler L, Devare S, Brennan CA. Confirmation of Putative HIV-1 Group P in Cameroon. J Virol, 2011, 85:1403–7
4. Villabona-Arenas CJ, Domyeum J, Mouacha F, Butel C, Delaporte E, Peeters M, Mpoudi-Ngole E, Aghokeng AF. HIV-1 group O infection in Cameroon from 2006 to 2013: Prevalence, genetic diversity, evolution and public health challenges. Infection, Genetics and Evolution, 2015, 36:210–6
5. Vallari A, Bodelle P, Ngansop C, Makamche F, Ndembi N, Mbanya D, Kaptué L, Gürtler LG, McArthur CP, Devare SG, Brennan CA. Four New HIV-1 Group N Isolates from Cameroon: Prevalence Continues to Be Low. AIDS Research and Human Retroviruses, 2010, 26:109–15
6. Semengue ENJ, Armenia D, Inzaule S, Santoro MM, Dambaya B, Takou D, Teto G, Nka AD, Yagai B, Fabeni L, Chenwi C, Angong Beloumou G, Djupsa Ndjeyep SC, Colizzi V, Perno C-F, Ceccherini-Silberstein F, Fokam J. Baseline integrase drug resistance mutations and conserved regions across HIV-1 clades in Cameroon: implications for transition to dolutegravir in resource-limited settings. Journal of Antimicrobial Chemotherapy, 2021, 76:1277–85
7. Nair M, Gettins L, Fuller M, Kirtley S, Hemelaar J. Global and regional genetic diversity of HIV-1 in 2010–21: systematic review and analysis of prevalence. The Lancet Microbe, 2024, 5
8. Alessandri-Gradt E, Moisan A, Plantier J-C. HIV-1 Non-Group M Strains and ART. Viruses, 2023, 15:780
9. Simon F, Mauclère P, Roques P, Loussert-Ajaka I, Müller-Trutwin MC, Saragosti S, Georges-Courbot MC, Barré-Sinoussi F, Brun-Vézinet F. Identification of a new human immunodeficiency virus type 1 distinct from group M and group O. Nat Med, 1998, 4:1032–7
10. Aghokeng AF, Mpoudi-Ngole E, Dimodi H, Atem-Tambe A, Tongo M, Butel C, Delaporte E, Peeters M. Inaccurate Diagnosis of HIV-1 Group M and O Is a Key Challenge for Ongoing Universal Access to Antiretroviral Treatment and HIV Prevention in Cameroon. PLOS ONE, 2009, 4:e7702
11. Mourez T, Lemée V, Delbos V, Delaugerre C, Alessandri-Gradt E, Etienne M, Simon F, Chaix M-L, Plantier J-C. HIV rapid screening tests and self-tests: Be aware of differences in performance and cautious of vendors. EBioMedicine, 2018, 37:382
12. Zouhair S, Roussin-Bretagne S, Moreau A, Brunet S, Laperche S, Maniez M, Barin F, Harzic M. Group O Human Immunodeficiency Virus Type 1 Infection That Escaped Detection in Two Immmunoassays. J Clin Microbiol, 2006, 44:662–5
13. Tebit DM, Patel H, Ratcliff A, Alessandri E, Liu J, Carpenter C, Plantier J-C, Arts EJ. HIV-1 Group O Genotypes and Phenotypes: Relationship to Fitness and Susceptibility to Antiretroviral Drugs. AIDS Res Hum Retroviruses, 2016, 32:676–88
14. Rodgers MA, Vallari AS, Harris B, Yamaguchi J, Holzmayer V, Forberg K, Berg MG, Kenmenge J, Ngansop C, Awazi B, Mbanya D, Kaptue L, Brennan C, Cloherty G, Ndembi N. Identification of rare HIV-1 Group N, HBV AE, and HTLV-3 strains in rural South Cameroon. Virology, 2017, 504:141–51
15. Fokam J, Salpini R, Santoro MM, Cento V, D'Arrigo R, Gori C, Perno CF, Colizzi V, Nanfack A, Gwom LC, Cappelli G, Takou D. Performance evaluation of an in-house human immunodeficiency virus type-1 protease-reverse transcriptase genotyping assay in Cameroon. Arch Virol, 2011, 156:1235–43
16. Katoh K, Standley DM. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Molecular Biology and Evolution, 2013, 30:772

17. Kalyaanamoorthy S, Minh BQ, Wong TK, Haeseler A von, Jermiin LS. ModelFinder: Fast Model Selection for Accurate Phylogenetic Estimates. Nature Methods, 2017, 14:587
18. Fabeni L, Berno G, Fokam J, Bertoli A, Alteri C, Gori C, Forbici F, Takou D, Vergori A, Zaccarelli M, Maffongelli G, Borghi V, Latini A, Pennica A, Mastroianni CM, Montella F, Mussini C, Andreoni M, Antinori A, Perno CF, Santoro MM. Comparative Evaluation of Subtyping Tools for Surveillance of Newly Emerging HIV-1 Strains. J Clin Microbiol, 2017, 55:2827–37
19. Liu TF, Shafer RW. Web Resources for HIV Type 1 Genotypic-Resistance Test Interpretation. Clin Infect Dis, 2006, 42:1608–18
20. Struck D, Lawyer G, Ternes A-M, Schmit J-C, Bercoff DP. COMET: adaptive context-based modeling for ultrafast HIV-1 subtype identification. Nucleic Acids Research, 2014, 42:e144
21. Pineda-Peña A-C, Faria NR, Imbrechts S, Libin P, Abecasis AB, Deforche K, Gómez-López A, Camacho RJ, de Oliveira T, Vandamme A-M. Automated subtyping of HIV-1 genetic sequences for clinical and surveillance purposes: Performance evaluation of the new REGA version 3 and seven other tools. Infection, Genetics and Evolution, 2013, 19:337–48
22. Pirkl M, Büch J, Friedrich G, Böhm M, Turner D, Degen O, Kaiser R, Lengauer T. Geno2pheno: recombination detection for HIV-1 and HEV subtypes. NAR Molecular Medicine, 2024, 1:ugae003
23. MINSANTE. DIRECTIVES NATIONALES SUR LA PRISE EN CHARGE DU VIH, 2021
24. Bibbins-Domingo K, Brubaker L, Curfman G. The 2024 Revision to the Declaration of Helsinki: Modern Ethics for Medical Research. *JAMA.* 2025;333(1):30–31.
25. Leoz M, Feyertag F, Kfutwah A, Mauclère P, Lachenal G, Damond F, Oliveira FD, Lemée V, Simon F, Robertson DL, Plantier J-C. The Two-Phase Emergence of Non Pandemic HIV-1 Group O in Cameroon. PLOS Pathogens, 2015, 11:e1005029
26. Mauclère P, Loussert-Ajaka I, Damond F, Fagot P, Souquières S, Monny Lobe M, Mbopi Keou FX, Barré-Sinoussi F, Saragosti S, Brun-Vézinet F, Simon F. Serological and virological characterization of HIV-1 group O infection in Cameroon. AIDS, 1997, 11:445–53
27. Ayouba A, Mauclère P, Martin PM, Cunin P, Mfoupouendoun J, Njinku B, Souquières S, Simon F. HIV-1 group O infection in Cameroon, 1986 to 1998. Emerg Infect Dis, 2001, 7:466–7
28. Vessière A, Rousset D, Kfutwah A, Leoz M, Depatureaux A, Simon F, Plantier J-C. Diagnosis and monitoring of HIV-1 group O-infected patients in Cameroun. J Acquir Immune Defic Syndr, 2010, 53:107–10
29. Oliveira FD, Mourez T, Vessiere A, Ngoupo P-A, Alessandri-Gradt E, Simon F, Rousset D, Plantier J-C. Multiple HIV-1/M + HIV-1/O dual infections and new HIV-1/MO inter-group recombinant forms detected in Cameroon. Retrovirology, 2017, 14:1
30. Peeters M, Gueye A, Mboup S, Bibollet-Ruche F, Ekaza E, Mulanga C, Ouedrago R, Gandji R, Mpele P, Dibanga G, Koumare B, Saidou M, Esu-Williams E, Lombart JP, Badombena W, Luo N, Vanden Haesevelde M, Delaporte E. Geographical distribution of HIV-1 group O viruses in Africa. AIDS, 1997, 11:493–8
31. Bekolo CE, Kouanfack C, Ateudjieu J, Bechem E, Ndeso S, Tendengfor N, Nsagha D, Choukem S. The declining trend in HIV prevalence from population-based surveys in Cameroon between 2004 and 2018: myth or reality in the universal test and treat era? BMC Public Health, 2023, 23:479
32. Ariën KK, Abraha A, Quiñones-Mateu ME, Kestens L, Vanham G, Arts EJ. The Replicative Fitness of Primary Human Immunodeficiency Virus Type 1 (HIV-1) Group M, HIV-1 Group O, and HIV-2 Isolates. Journal of Virology, 2005, 79:8979
33. Lemey P, Pybus OG, Rambaut A, Drummond AJ, Robertson DL, Roques P, Worobey M, Vandamme A-M. The Molecular Population Genetics of HIV-1 Group O. Genetics, 2004, 167:1059–68
34. Hemelaar J. The origin and diversity of the HIV-1 pandemic. Trends in Molecular Medicine, 2012, 18:182–92
35. D'arc M, Ayouba A, Esteban A, Learn GH, Boué V, Liegeois F, Etienne L, Tagg N, Leendertz FH, Boesch C, Madinda NF, Robbins MM, Gray M, Cournil A, Ooms M, Letko M, Simon VA, Sharp PM,

Hahn BH, Delaporte E, Mpoudi Ngole E, Peeters M. Origin of the HIV-1 group O epidemic in western lowland gorillas. Proceedings of the National Academy of Sciences, 2015, 112:E1343–52

36. Gautheret-Dejean A, Mesmin-Poho S, Birguel J, Lemée V, Huraux J-M, Plantier J-C. Unequal Detection of HIV Type 1 Group O Infection by Simple Rapid Tests. Clinical Infectious Diseases, 2008, 46:1936–7
37. Stoffels K, Vanroye F, Mortier V, Debaisieux L, Delforge M-L, Depypere M, Dessilly G, Vaira D, Vancutsem E, Van den Wijngaert S, Van Laethem K, Vercauteren KOA, Verhofstede C, Fransen K. Chronic and Early Antiretroviral Therapy Impact Human Immunodeficiency Virus (HIV) Serological Assay Sensitivity, Leading to More False-Negative Test Results in HIV Diagnosis. The Journal of Infectious Diseases, 2020, 222:1660–9
38. Mbouyap PR, Fokam J, Ngoufack Jagni Semengue E, Mossiang L, Takou D, Ambe Chenwi C, Nka AD, Dambaya B, Teto G, Angong Beloumou G, Djupsa Ndjeyep SC, Ka'e AC, Kouanfack C, Ndjolo A, Mbopi Keou F-X. HIV-1 genotypic profiling ensures effective response to third-line antiretroviral therapy in Cameroon. Medicine (Baltimore), 2023, 102:e33897
39. Luk K-C, Kaptué L, Zekeng L, Soriano V, Gürtler L, Devare SG, Schochetman G, Hackett J. Naturally Occurring Sequence Polymorphisms within HIV Type 1 Group O Protease. AIDS Research and Human Retroviruses, 2001, 17:1555–61
40. Depatureaux A, Charpentier C, Leoz M, Unal G, Damond F, Kfutwah A, Vessière A, Simon F, Plantier J-C. Impact of HIV-1 Group O Genetic Diversity on Genotypic Resistance Interpretation by Algorithms Designed for HIV-1 Group M. JAIDS Journal of Acquired Immune Deficiency Syndromes, 2011, 56:139
41. Fokam J, Chenwi CA, Tala V, Takou D, Santoro MM, Teto G, Dambaya B, Anubodem F, Semengue ENJ, Beloumou G, Djupsa S, Assomo E, Fokunang C, Alteri C, Billong S, Bouba NP, Ajeh R, Colizzi V, Mbanya D, Ceccherini-Silberstein F, Perno C-F, Ndjolo A. Pre-Treatment HIV Drug Resistance and Genetic Diversity in Cameroon: Implications for First-Line Regimens. Viruses, 2023, 15:1458
42. Kandel CE. Dolutegravir – a review of the pharmacology, efficacy, and safety in the treatment of HIV. Drug Des Devel Ther, 2015, 9:3547–55
43. Eaton JW, Sands A, Barr-DiChiara M, Jamil MS, Baggaley R, Barr BAT, Kalua T, Jahn A, Maheu-Giroux M, Johnson CC. Accuracy and performance of the WHO 2015 and WHO 2019 HIV testing strategies across epidemic settings, 2021:2021.03.31.21254700
44. Eaton JW, Terris-Prestholt F, Cambiano V, Sands A, Baggaley RC, Hatzold K, Corbett EL, Kalua T, Jahn A, Johnson CC. Optimizing HIV testing services in sub-Saharan Africa: cost and performance of verification testing with HIV self-tests and tests for triage. J Int AIDS Soc, 2019, 22:e25237

556 **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	04/08/2021	1431	46
GO-CAM-62	45	Female	01/01/2005	West	04/01/2018	5235	443
GO-CAM-95	45	Male	01/01/2014	Centre	22/10/2018	13,8410	57
GO-CAM-75	26	Female	01/01/2008	West	14/08/2018	58,900	39
GO-CAM-020	35	Male	10/04/2006	Centre	14/05/2019	5446	228
GO-CAM-29	53	Male	01/06/2005	Centre	11/01/2022	86,464	U/A
GO-CAM-94	34	Female	01/01/2011	Centre	11/01/2022	18,456	U/A
GO-CAM-02	40	Female	26/07/2016	East	04/02/2022	6314	28
GO-CAM-83	55	Male	In 2010	Centre	15/09/2022	U/A	U/A

557 ID, identification; U/A, unavailable; GRT, genotypic resistance test.

558

559

560

561

562

563

564

565

566 **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%)

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

569

570

571

572

573

574

575

576

577

578 **Table 3: Results of rapid diagnostic tests on samples with *group O* strains.**

Sample ID	First Response HIV1-2.O	One Step	Determine HIV1/2 set (Abbot)	KHB colloidal gold	ACRO Rapid Test (HIV1/2 & 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]

579 ID, identification; R, reactive; R1, reactive to HIV-1; NR, non-reactive; *This analysis was carried out on the 8 available
580 remnant plasma aliquots. In bold are unreactive results.

581

582

583

584

585

586

587

588

589 **Table 4: Molecular characterization of reactive biomarkers in the 8 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

590 ID, identification; UND, undetermined; POS, positive. In bold are undetermined results.

591

592

593

594

595

596

597

598

599

600

601