

Analysis of antibodies involved in cases of discordant and indeterminate HIV serology in Togo from 2016 to 2018

Abstract

Introduction: The national algorithm for the diagnosis of HIV infection in Togo leads sometimes to discordant or indeterminate results. The objective of this study was to explore the characteristics of discordant and indeterminate HIV serologies at the National Reference Center for HIV testing (CNR-VIH) in Togo.

Material and methods: Through a cross-sectional study, we analyzed the cases of discordant and indeterminate serologies from 2016 to 2018 at the CNR-VIH. Three kind of tests were used. The first one is a screening test also called “test 1” is very sensitive while the confirmatory test also called “test 2” is very specific and used when the sample is reactive with test 1. The third test is an immunoblotting test which is used in case of discordance between test 1 and test 2 results. We used Vironostika HIV Uni-Form II Ag/Ab (BioMérieux, Geneva, Switzerland), Determine Alere HIV-1/2 Serum/Plasma (Chiba, Japan) and Murex HIV Ag/Ab (Dartford, UK) as test 1 while Tri-Dot HIV-I/II (J. Mitra & Pvt Ltd. New Delhi-110-India Co.) and Inno-Lia HIV-I/II Score (Fujirebio, Ghent, Belgium) were used respectively as test 2 and test 3. The test 3 allowed us to determine the antibodies involved in the occurrence of indeterminate serologies. A pool of indeterminate samples was tested for qualitative detection of HIV-1 RNA by RT-PCR using the NucliSENS EasyQ HIV-1 platform from BioMérieux.

Results: A total of 555 discordant serologies corresponding to 4.3% of all serologies performed over the 3 years have been analyzed. The average age of the subjects tested was 36.9 ± 17.4 years and men were slightly more represented with a sex ratio of 1.03. Of the 555 samples, 81 (14.7%) were reactive with 53 (9.6%) cases of HIV-1 and 28 (5.1%) cases of HIV-2. Two hundred and thirty-one (41.6%) samples were negative while the remaining 243 (43.8%) were indeterminate. The frequencies of antibodies (Ab) against gp120, gp41, p24, p31, p17, gp105 and gp36 were respectively 10.3%, 46.3%, 15.5 %, 11.2%, 8.8%, 5.4% and 5.0%. Among the 257 cases for which gp41 Ab had been detected, 202 (78.6%) were cases of indeterminate serology with the only presence of gp41 Ab. Qualitative HIV-1 RNA testing on 71 (29.2%) indeterminate samples was negative.

Conclusion: This study showed that discordant serologies are frequently found in our common practice in Togo. The use of immunoblotting tests has the advantage of making it possible to elucidate more than half of these discordant serologies. The contribution of molecular biology techniques is uncertain. The large majority of these indeterminate serologies are due to cross-reactions particularly with gp41. These indeterminate serologies require not only further studies for their understanding but also an update of HIV diagnosis algorithm in Togo.

Keywords: HIV, discordant serologies, indeterminate serologies, RT-PCR, glycoprotein-41

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Malewe Kolou,^{1,2} Charnelle Ingrid Kengne Tegue,¹ Liza Koboyo Nadjir,^{1,3} Amivi Amenyah-Ehlan,² Komlan Ali-Edje,² Alassane Ouro-Medeli,² Mensah Douffan,² Mounerou Salou,^{1,2} Anoumou Dagnra^{1,2}

¹Faculty of Health Sciences, University of Lome (FSS-UL), Togo

²National Reference Center for HIV testing (CNR-VIH), Togo

³National Blood Transfusion Center (CNTS), Togo

Correspondence: Malewe Kolou, Faculty of Health Sciences, University of Lome (FSS-UL), Togo, 13 BP 378 Lome, Togo, Tel +228 90106600, Email koloumalewe@hotmail.fr

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Abbreviations: Ab, antibody; AIDS, acquired immuno deficiency syndrome; CNR-VIH, national reference center for HIV testing; DNA, deoxyribonucleic acid; ELISA, enzyme-linked immunosorbent assay; gp, glycoprotein; HIV, human immunodeficiency virus; RDTs, rapid diagnostic tests; RNA, ribonucleic acid; RT-PCR, reverse transcriptase polymerase chain reaction; STI, sexually transmitted infection ; WHO, world health organization

Introduction

The human immunodeficiency virus (HIV) infection remains a great public health problem particularly in low income countries.^{1,2} The diagnosis of this infection, which is clinical, virological and serological, is the first and one of the main axes of response against the disease.³ The WHO recommends for serological diagnosis, the

use of rapid diagnostic tests (RDTs) in low-income countries.⁴⁻⁶ Thus, the wide distribution of RDTs in these countries and their use out of accredited medical laboratories raises questions about their reliability in terms of sensitivity and specificity.⁷⁻¹⁰ Consequently, the use of these tests is often based on algorithms combining several tests.^{11,12} In Togo, the algorithm for the biological diagnosis of HIV infection in adults and children over 18 months requires for the performing of a screening test called “test 1” which must be confirmed by a “test 2” if the first test is reactive. Serology is said to be discordant when the screening test is reactive while the confirmatory test is negative. In case of discrepancy between test 1 and test 2, it is necessary to carry out a “test 3” to clarify the case. This test 3 may however not be conclusive. In this case, the serology is qualified as indeterminate.¹³ Thus, the serological status of some people towards HIV is difficult to define. That is why we carried out this study which objective was

to explore the characteristics of discordant and indeterminate HIV serologies at the National Reference Center for HIV tests (CNR-VIH) in Togo, focusing on the expression of HIV antibodies that are involved.

Material and methods

Setting and study design: Through a cross-sectional study, we have analyzed the cases of discordant HIV serologies, characterized by a reactive screening test (test 1) and a negative confirmation test (test 2), over a period of 3 years from January 1, 2016 to December 31, 2018. The study took place at the CNR-VIH which is the national reference laboratory for HIV testing. The CNR-VIH is located in Lome, the capital city of Togo, and receives samples from all the health structures in the country in case of difficulties during the interpretation of the tests for HIV diagnosis. These samples come from patients suspected of having an HIV infection or from subjects received for voluntary screening or for a medical fitness because of administrative reasons. The CNR-VIH is an operational entity of the National Program against AIDS and Sexual Transmitted Infections (STIs) in Togo. Moreover, the CNR-VIH is installed in the Sylvanus Olympio Teaching Hospital which is the biggest health structure in Togo. As such, the CNR-VIH provides routine biological diagnosis of HIV for patients from this hospital.

Determination of HIV serological status: HIV serological status was obtained using the national algorithm for biological diagnosis of HIV infection in adults and children over 18 months, which includes three different tests.¹⁴ Test 1, also called screening test, is very sensitive with a risk of false positive results. Three different types of test 1 were used in this study. These were Vironostika HIV Uni-Form II Ag/Ab (BioMérieux, Geneva, Switzerland), Determine Alere HIV-1/2 Serum/Plasma (Chiba, Japan) and Murex HIV Ag/Ab (Dartford, UK). These tests had a sensitivity of 100% and a specificity between 97.96% and 100%. Test 2, also called confirmation test, is a very specific immunoassay which is usually capable of discriminating HIV-1 infection from HIV-2 infection. During our study period, the Tri-Dot HIV-1/II (J. Mitra & Pvt Ltd. New Delhi-110-India Co.) was the only type 2 test used at CNR-VIH. Its sensitivity and specificity were 100%. Test 3 is an immunoblotting test and Inno-Lia HIV-I/II Score (Fujirebio, Ghent, Belgium) was the one we used during our study. When a sample was received to elucidate a discrepancy, the entire process from test 1 was repeated at the CNR-VIH. All these tests were carried out by scrupulously following the procedures provided by the manufacturers.

Detection of HIV-1 RNA: In order to assess the contribution of the molecular tool, a pool of samples whose serological status remained undetermined after test 3 and which had been collected for less than 24 hours have been tested for qualitative detection of HIV-1 RNA.

The Bio Mérieux's NucliSENS EasyQ HIV-1 platform was used for this purpose.

Statistical analysis: SPSS version 2.5 software was used for statistical analysis. The comparison of frequencies was carried out using the Chi-square test. Fisher's exact test is used when counts less than 5 are analyzed. A p-value of less than 0.05 was considered as statistically significant.

Results

General characteristics of the study population: Between 2016 and 2018, a total of 12,907 HIV serologies were carried out at the CNR-VIH in Togo. Among these serologies, 555 (4.3%) were discordant after tests 1 and 2. The frequencies of discordant serologies were respectively 4.8%, 3.0% and 5.5% for 2016, 2017 and 2018. The following results will concern the 555 discordant cases. The Vironostika HIV Uni-Form II Ag/Ab test was the most used in 62.7% (n=348) of cases versus 34.8% and 2.5% respectively for Murex HIV Ag/Ab and Determine Alere HIV-1/2. The average age of the subjects concerned by the discordant tests was 36.9 ± 17.4 years with a median of 36 years. The extreme ages were 1 year and 90 years. The 25-49 age group was the most represented in our study with a frequency of 77.71% of the study population. There were 284 male subjects corresponding to a sex-ratio of 1.03.

Results of test 3: Of the 555 discordant samples analyzed using Inno-Lia HIV-I/II Score, 81 (14.7%) were reactive with 53 cases of HIV-1 (9.6%) and 28 cases of HIV-2 (5.1%). HIV-2 accounted for 34.6% of the 81 reactive cases. Two hundred and thirty-one (41.6%) samples were negative while 243 (43.8%) samples remained indeterminate. Figure 1 shows the distribution of discordant cases according to the result of test 3 and the sex of the subjects tested.

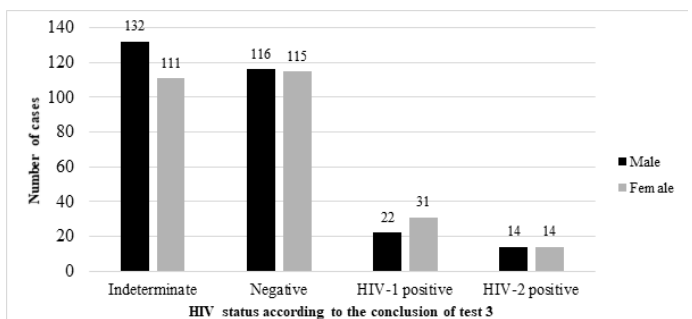


Figure 1 Distribution of results of test 3 by gender.

As shown in Table 1, the frequencies of antibodies (Ab) against gp120, gp41, p24, p31, p17, gp105 and gp36 were respectively 10.3% (n=57), 46.3% (n=257), 15.5% (n=86), 11.2% (n=62), 8.8% (n=49), 5.4% (n=30) and 5.0% (n=28).

Table 1 Frequency of HIV antibodies in the 555 cases of discordant serology according to the result of test 3

	gp120 Ab		gp41 Ab		Ab p24		p31 Ab		p17 Ab		gp105 Ab		gp36 Ab	
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
Indeterminate	14	(24,6)	202	(78,6)	13	(15,1)	10	(16,1)	10	(20,4)	2	(6,7)	1	(3,6)
Negative	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HIV-1 positive	39	(68,4)	49	(19,1)	47	(54,7)	29	(46,8)	37	(75,5)	0	0	0	0
HIV-2 positive	4	(7,0)	6	(2,3)	26	(30,2)	23	(37,1)	2	(4,1)	28	(93,3)	27	(96,4)
Total	57	-100	257	-100	86	-100	62	-100	49	-100	30	-100	28	-100

The distribution of the 243 cases of indeterminate serology according to the 3 years of study was 68 (29.4%), 60 (26.0%) and

103 (44.6%) respectively for 2016, 2017 and 2018. Among the 257 cases for which gp41 Ab had been detected, 202 (78.6%) were cases

of indeterminate serology with the only gp41 Ab. There was no statistically significant difference in the expression of these gp41 Ab according to gender and age.

Results of HIV-1 RNA detection: Among the 243 samples whose result remained indeterminate after the immunoblotting test, 71 (29.2%) were used for qualitative detection of HIV-1 RNA. For all of these 71 samples, the search for the viral genome was negative.

Discussion

In Togo, the biological diagnosis of HIV infection is done according to an algorithm including a combination of 3 types of tests. The tests qualified as “test 1” also called “screening tests” are very sensitive and often do not discriminate HIV-1 from HIV-2. These are generally 4th generation ELISA tests or RDTs. Their sensitivity is increased because of the combined detection of HIV antigens and antibodies. Tests referred to as “test 2” or “confirmation tests” are very specific and capable to distinguish HIV-1 from HIV-2. These are usually RDTs. The tests referred to as “test 3” are immunoblots that make it possible to distinguish antibodies against multiple antigens of HIV-1 and HIV-2. These three types of tests are not always able to solve all the problems related to the diagnostic difficulties of HIV infection.

During the 3 years covered by this study, 555 discordant serologies have been processed at the CNR-VIH, corresponding to a frequency of 4.3% of all serological tests carried out. This rate of discordant serologies is probably higher than the national rate of discordance insofar as the CNR-VIH receives and analyzes several discordant samples from other laboratories in the country which do not have the expertise in the use of immunoblot tests. Indeed, the interpretation of the results of immunoblot-type tests is often difficult and requires adequate training of users. Thus, to date, only regional laboratories are able to use immunoblot-type tests. These difficulties have led some manufacturers to offer interpretation software, as is the case for the Inno-Lia HIV-I/II Score test which can be interpreted by using the LiRASTM software. To elucidate the cases of discrepancy, the samples received at the CNR-VIH are again tested according to the national algorithm starting from test 1. The national reference system makes the samples come from the whole country. This could explain the high rate of discordant serologies that we report. In a study carried out at Bobo-Dioulasso in Burkina-Faso, D. Kania reported a prevalence of 1.3% of discordant tests between 2005 and 2007 among pregnant women included in the Prevention of Mother-To-Child Transmission (PMTCT) program.¹⁵

The Vironostika HIV Uni-Form II Ag/Ab test, a 4th generation sandwich-type combined ELISA test, was the most used as test 1 in 62.7% of cases. All samples were tested with Tri-Dot HIV-1/2 as test 2 and with Inno-Lia HIV-I/II Score as test 3. This last test elucidated 56.2% (n=312) cases of the 555 discordant serologies. The Inno-Lia HIV-I/II Score test is an immunoblot using recombinant proteins and synthetic peptides from HIV-1 and HIV-2. The wide-scale use of this type of test has increased the sensitivity and specificity of HIV antibodies detection compared to the classic Western Blot which uses native viral antigens.

Among the 81 cases of discordant serologies which were reactive to Inno-Lia HIV-I/II Score test, 53 (65.4%) were cases of HIV-1 infection versus 28 (34.6%) cases of HIV-2 infection. Normally, the prevalence of HIV-2 is very low. The most important prevalence found in Guinea-Bissau was 9%.¹⁶ Thus, the high proportion of 34.6% of HIV-2 among the cases of discordant serologies indicates that the confirmation test (test 2) has a poor performance in the detection of

HIV-2 cases. This situation must be seriously taken into account as Togo is a Western African country where HIV-2 is endemic.¹⁷ It would therefore be interesting to have new tests that are more sensitive for HIV-2 antibodies in order to reduce the frequency of discordant and indeterminate serologies. Apart from the problem of identifying the serological status, HIV-2 infection also suffers from the insufficiency of molecular tests making difficult not only the virological monitoring by measuring the viral load but also the early diagnosis in children born to HIV-2-infected mothers. Another fact that may trouble the togolese scientific community is that unlike HIV-1, HIV-2 infection has shown a stable trend in Togo for several years without a downward trend. Indeed, according to a study carried out at the CNR-VIH concerning the monitoring of the evolution of HIV infection over 10 years, the prevalence of HIV-2 fluctuated between 0.07 and 0.39% from 2005 to 2014.¹⁸ In fact, HIV-2 infection did not experience the decline observed for HIV-1 during this decade. While the overall prevalence of HIV infection in the general population fell from 4.1% in 2004 to 2.5% in 2014, a spectacular drop in HIV prevalence was observed for CNR-VIH from 35.3% to 13.9%.¹⁹ All these data lead us to believe that HIV-2 deserves greater attention in terms of diagnosis and follow-up.

Although gp120 and gp41 are considered specific for HIV-1, antibodies directed against these 2 envelope glycoproteins were detected by the immunoblot test 3 respectively in 4 and 6 subjects out of the 28 who were infected with HIV-2. The gp105 and gp36 envelope antigens were on the other hand sufficiently specific for HIV-2 since 100% of the gp105 antibodies and 96.4% of the gp36 antibodies were detected in subjects with HIV-2. Regarding p31, p24 and p17 antibodies, they were found at various frequencies in subjects with both HIV-1 and HIV-2, indicating the existence of cross-reactivities between these antigens which are shared by the 2 types of virus. No cases of co-infection were found in our study.

Among the 257 cases of reactive gp41 bands revealed by Inno-Lia HIV-I/II Score, 202 (78.6%) were cases of indeterminate serology with the only presence of gp41 antibodies. These isolated gp41 antibodies were responsible for 83.1% of the 243 cases of indeterminate serology. This high proportion of the involvement of gp41 antibodies was also reported in Mali by B. Nouhoum where the gp41 viral band was found in 80% of cases.¹⁹ The work of Al-Kindi et al. in Oman over the same period of study as ours also showed a strong involvement of gp41 antibodies in the occurrence of undetermined serological status in expatriate Africans.²⁰ It is in fact very likely to be cross-reactions with antibodies against other microorganisms such as schistosomes or Plasmodium or the Epstein Barr virus or other antigens not identified to date including auto-antigens.^{21,22} Another hypothesis mentioned to explain these cases of indeterminate serologies is the existence of a real HIV infection which resulted in the spontaneous elimination of the virus by the subject without necessity of antiretroviral treatment. However, further investigations are necessary to better elucidate the cases of isolated gp41 antibodies.

The wide-scale use of nucleic acid amplification tests, particularly the PCR, leads to think that the bias associated with serological tests for detecting HIV infection could be reduced.²³ That is why we carried out a qualitative search of the viral genome by RT-PCR in 71 subjects whose serological status was undetermined. The PCR test was negative for all these samples. However, the PCR test allowed us to remove a doubt on the meaning of these indeterminate HIV serologies. Indeed, the absence of HIV-1 RNA suggests that it is not an HIV-1 infection insofar as the subjects concerned were not on antiretroviral treatment. Normally, people living with HIV who are not on antiretroviral therapy people living with HIV who are not on antiretroviral therapy should

have high viral loads. The combination of proviral DNA detection could also increase the sensitivity of molecular tests. In this context, and knowing that a large proportion of indeterminate serologies are linked to HIV-2, there remains the problem of the low availability of molecular tests for this type of virus. This may be responsible for the escape of some HIV-2 cases from diagnosis.

If indeterminate serologies are most of the time due to false reactive results, an HIV status that is neither positive nor negative makes decision-making difficult in certain situations, plunging the actors into a real dilemma^{20,24} and leading sometimes to wrong decisions.²⁵ The psychological weight of such a situation is quite heavy both for the people tested and for the actors who have to make a decision with regard to the serological status. This difficulty is even greater when the HIV serology is carried out on several times and that it always remains undetermined even though it is done with the aim of medical fitness assessment counting for a prenuptial agreement or an administrative purpose such as visa applications or bank loans.²⁰ The impossibility of concluding is often a source of a certain stigmatization of the concerned subjects. Possible solutions to limit the impact of these indeterminate serologies go not only through the development of more efficient tests, but also through the definition of new algorithms for HIV infection screening in Togo.²⁶

Conclusion

Discordant HIV serologies represent a significant part of the CNR-VIH activities. The use of immunoblot-type tests to elucidate these discordant cases is not easy because of the difficulties of interpretation. This study showed that the confirmatory serological tests available to detect HIV-2 infections are less well than those of HIV-1. Beyond the strategies for better detecting cases of HIV-2, it is urgent to also address the question of indeterminate HIV serologies in the sense of the search for possible cross-reactions in particular with the gp41 antigen which is responsible for more than three quarters of undetermined cases. In addition to the search for more efficient tests, it is appropriate to consider a revision of the screening algorithm for HIV infection in Togo.

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Conflicts of interest

The authors declare no conflict of interest.

References

1. WHO. HIV and Aids. 2022.
2. UNAIDS. Global HIV statistics. Fact sheet. 2022.
3. WHO. Consolidated guidelines on HIV prevention, testing, treatment, service delivery and monitoring: recommendations for a public health approach. 2021.
4. Kosack CS, Page AL, Beelaert G, et al. Towards more accurate HIV testing in sub-Saharan Africa: a multi-site evaluation of HIV RDTs and risk factors for false positives. *AIDS Society*. 2017;20(1):21345.
5. Foglia G, Royster G, Wasunna K, et al. Use of rapid and conventional testing technologies for human immunodeficiency virus type 1 serologic screening in a rural Kenyan reference laboratory. *J Clin Microbiol*. 2004;42(8):3850–3852.
6. Salou M, Dagnra AY, Mlaga KD, et al. Evaluation de la performance de trois tests de diagnostic de l'infection à VIH à Lomé (Togo). *Revue Bio-Africa*. 2010;(8):7–12.
7. Adetunji AA, Kuti MA, Audu RA, et al. Discordant rapid HIV tests: lessons from a low-resource community. *HIV Med*. 2018;19(1):72–76.
8. Dagnra A, Dossim S, Salou M, et al. Evaluation of 9RDT for screening HIV infection in Lomé, Togo. *Med Mal Infect*. 2014;44(11–12):525–529.
9. Gray RH, Serwadda D, Lutalo T, et al. Limitation of rapid HIV-1 tests during screening for trials in Uganda: diagnostic test accuracy study. *Bmj*. 2007;335(7612):188–190.
10. Klarkowski DB, Wazome JM, Lokuge KM, et al. The evaluation of a rapid in situ HIV confirmation test in a program with high failure rate of the WHO HIV two-test diagnostic algorithm. *Plos One*. 2009;4(2):e4351.
11. Mayhood M, Afwamba C, Odhiambo O, et al. Validation, performance under field conditions, and cost-effectiveness of capillus HIV-1/HIV2 and determine HIV-1/2 rapid human immunodeficiency virus antibody assays using sequential and parallel testing algorithms in Tanzania. *J Clin Microbiol*. 2008;46(12):3946–3951.
12. Srichatrapimuk S, Setthaudom C, Apiwatanakul N, et al. Anti-HIV serological test algorithms to reduce false-positive and inconclusive results for low HIV prevalence and resource-limited areas. *Int J STD AIDS*. 2022;33(1):63–72.
13. Alexander TS. Human immunodeficiency virus diagnostic testing: 30 years of evolution. *Clin Vaccine Immunol*. 2016;23:249–253.
14. Programme National de Lutte contre le Sida et les IST. Rapport Annuel d'activités. 2014
15. Dramane K. Sérologies indéterminées par l'utilisation des tests rapides pour le diagnostic de l'infection à VIH chez les femmes enceintes à Bobo-Dioulasso, Burkina Faso. Mémoire de Diplôme d'Etudes Approfondies. Burkina Faso : Université polytechnique de Bobo-Dioulasso. 2010.
16. Schim van der Loeff MF, Aaby P. Towards a better understanding of the epidemiology of HIV-2. *AIDS*. 1999;(13 Suppl A):S69–S84.
17. Ba S, Dia-Badiane NM, Hawes SE, et al. HIV-2 infection in Senegal: virological failures and resistance to antiretroviral drugs (ARVs). *The Pan African Medical Journal*. 2019;33:222.
18. Amenyah-Ehlan A, Salou M, Kolou M, et al. Trends in HIV-2 Seroprevalence at the National Reference Center of HIV from 2005 to 2014 in Lomé, Togo. *World journal of AIDS*. 2017;7:239–246.
19. Nouhoum B. Prévalence comparée des infections VHC et VIH au Mali. Mémoire en Sciences Biomédicales et Pharmaceutiques. 2012-2013: Université de Liège.
20. Al-Kindi H, Al-Jardani A. HIV serology false positivity among expatriates from Africa: a screening dilemma. *Journal of Medical Microbiology* 2020;69(6):812–816.
21. Reid J, Van Zyl G, Linström M, et al. High positive HIV serology results can still be false positive. Case report. *IDCases*. 2020;21 :e00849.
22. Elm J, Desowitz R, Diwan A. Serological cross-reactivities between the retrovirus HIV and HTLV-1 and the malaria parasite Plasmodium Falciparum. *P N G Med*. 1998;41(1):15–22.
23. Michaeli M, Wax M, Gozlan Y, et al. Evaluation of Xpert HIV-1 Qual assay for resolution of HIV-1 infection in samples with negative or indeterminate Geenius HIV-1/2 results. *Journal of Clinical Virology*. 2016;76:1–3.

24. Yuksel P, Saribas S, Kuskucu M, et al. Problems encountered in conventional HIV 1/2 Algorithms: lack of necessity for immunoblot assays to confirm repeated ELISA reactive results. *Afri Health Sci.* 2018;18(2):407–416.
25. Ochola J, Imbach M, Eller LA, et al. False reactive HIV-1 diagnostic test results in an individual from Kenya on multiple testing platforms-A case report. *IDCases.* 2021;23:e01035.
26. Linley L, Ethridge SF, Oraka E, et al. Evaluation of supplemental testing with the Multispot HIV-1/HIV-2 Rapid Test and APTIMA HIV-1 RNA Qualitative Assay to resolve specimens with indeterminate or negative HIV-1 Western blots. *Journal of Clinical Virology.* 2013;58(Suppl 1):e108–e112.