# Inconclusive results with HIV serodiagnosis algorithms, and HIV-1 and HIV-2 co-infection in North-eastern Democratic Republic of Congo

**Serge Tonen-Wolyec**[1], **Salomon Batina Agasa**[1] , **Esperance Kumbati**[2], **Alliance Tagota**,[3]**,**
**Jacques Ossinga Bassandja**[1], **Charles Kayembe Tshilumba**[1], **and Laurent Bélec**[4]

[1]Faculty of Medicine and Pharmacy, University of Kisangani, Kisangani, Democratic Republic of the Congo;
[2]Provincial Reference Laboratory of AIDS and STI, Kisangani, Democratic Republic of the Congo;
[3] Provincial AIDS & STI Control Programme, Kisangani, Democratic Republic of the Congo;
[4]Laboratory of virology, Hôpital Européen Georges Pompidou and Faculty of Medicine, Paris Descartes University, Paris, France.

**Abstract**

**Background:** Inconclusive serodiagnosis of HIV infection is particularly frequent in Central Africa. The aims of this study were to: (i) determine the rate of inconclusive results with the two-test algorithm that the WHO proposed in 1997 (WHO II) versus the three-test algorithm (revised in 2012 and consolidated in 2015 by WHO) for HIV testing, and (ii) determine the prevalence of HIV-1 and HIV-2 co-infection in the north-eastern region of the Democratic Republic of the Congo (DRC).

**Methods:** A multicentre cross-sectional study was performed between March and June 2016 in Kisangani and Bunia, the capital cities of Tshopo and Ituri provinces respectively. Alere Determine™ HIV-1/2 (Alere Medical Co. Ltd., Japan), Uni-Gold™ HIV (Trinity Biotech Manufacturing Ltd., Ireland) and recomLine HIV-1 and HIV-2® IgG (Biosynex, France) were the first, second and third tests in the serial algorithm.

**Results:** The rate of inconclusive results was 1.1% (95% CI: 0.4 – 3.1) with the two-test algorithm and 0.4% (95% CI: 0.1 – 2.1) with the three-test algorithm (p< 0.001). The prevalence of HIV-1 and HIV-2 co-infection among HIV positive sera was 16.7% (95% CI: 4.7 – 44.8).

**Conclusion:** The three-test algorithm HIV testing strategy significantly reduces the rate of inconclusive results. In addition, the prevalence of HIV-1 and HIV-2 co-infection is higher in a context where HIV-2 infection is poorly documented. Large-scale research is essential to clarify these results.

## Introduction

Access to HIV diagnosis can be life-saving and essential to the success of the HIV response. This is because HIV testing services are the gateway to treatment, prevention and care. The WHO algorithm, which uses rapid diagnostic tests (RDT) in series to diagnose HIV, has been critical in scaling up access to life-saving treatment [1]. However, this algorithm is prone to increase inconclusive results. There is abundant literature about the concern regarding inconclusive sera on the serodiagnosis of HIV infection in sub-Saharan Africa [2, 3].

Email: wolyec@gmail.com

In principle, during the serodiagnosis, serum should respond, regardless of the test used, be it negative (absence of specific anti-HIV antibodies) or positive (presence of specific anti-HIV antibodies). Serum can be described as "inconclusive" (neither positive nor negative) when the test result is uninterpretable or when discrepant results are obtained from different tests within a given algorithm. Despite the increase in the quality of serodiagnostic tests, diagnosis of HIV infection remains difficult in Central Africa because of many cross-reactions [3].

With an estimated HIV prevalence of 1.2% among people aged 15 to 49 years, the Democratic Republic of the Congo (DRC) is considered a low HIV prevalence country [4]. However, data on the rates of HIV-1 and HIV-2 co-infection are scant and fragmentary. Although the WHO recommendations for HIV testing in resource-limited settings is widely used, few studies have investigated the accuracy of different WHO algorithms and the importance of HIV-1 and HIV-2 co-infection that impact on antiretroviral management.

The aims of this study were to: (i) determine the rate of inconclusive results with the two-test algorithm that the WHO proposed in 1997 (WHO II) versus the three-test algorithm (revised in 2012 and consolidated in 2015 by WHO) for HIV testing, and (ii) determine the prevalence of HIV-1 and HIV2 co-infection in north-eastern region of the DRC.

**Material and methods**

We conducted this multicenter, cross-sectional study between March and June 2016 in Kisangani and Bunia, the capital cities of Tshopo and Ituri provinces respectively. Participants were selected from five Voluntary HIV Counselling Testing (VCT) sites at the health centres of Neema and Boyoma in Kisangani. The VCT of Bunia included the *Hôpital Général de Référence de Bunia*, the *Centre hospitalier Bunia cité* and the *Clinique Mpabenda*. Inclusion criteria were as follows: male or female gender, at least 18 years old and willing to give advanced written consent to undergo HIV testing. The exclusion criteria were age < 18 years, and unwillingness to follow protocol instructions. Sociodemographic characteristics of participants were published in our previous paper [5]. Serological HIV testing at the VCT sites was first carried out as per the national algorithm of the DRC Ministry of Public Health, based on a WHO II twotest algorithm [6]. First, 50 µl of whole blood was serologically tested for HIV-1/HIV-2 antibodies in series using Alere Determine™ HIV-1/2 (Alere Medical Co. Ltd., Matsudo-shi, Chiba-ken, Japan). The second test used Uni-Gold™ HIV (Trinity Biotech Manufacturing Ltd., Bray, Co. Wicklow, Ireland). HIV serological results were negative if the first HIV test was negative. The results were positive if both HIV tests were positive; the tests were inconclusive if the tests yielded discordant results. After the tests, blood was drawn by venipuncture in EDTA tubes from each participant. Blood was then centrifuged at 1000 rpm for 15 minutes; plasma was aliquoted and kept frozen at -4° C until used at the Reference Provincial Laboratory of Kisangani. All samples were re-tested on plasma in the Reference Laboratory, using Alere Determine™ HIV-1/2 (Alere Medical Co. Ltd.) and Uni-Gold™ HIV (Trinity Biotech Manufacturing Ltd.) as the first and second tests, respectively. A third and final HIV

testing for confirmation was done using recomLine HIV-1 and HIV-2® IgG (Biosynex, Strasbourg, France), as per the 2012-revised and 2015consolidated WHO strategy for HIV testing [7,8]. HIV serological results were negative if the first HIV tests were negative, positive if the three were positive or inconclusive when the tests yielded discordant results. It should be noted that the third test has the ability to discriminate against HIV-1 and HIV-2. Manufacturers' instructions for test procedures were followed.

The study was conducted after obtaining ethical approval from the Ethics Committee of the School of Public Health of the University of Kinshasa. Informed consent was obtained from all volunteers in writing. No personal information from the participants was registered to ensure anonymity. All data were first entered into an Excel file, then analyzed using SPSS 20.0 (Chicago, IL). Descriptive statistics were computed. The results were presented with 95% confidence interval (CI) using the Wilson score bounds. Fisher's exact test was used to test the difference between the inconclusive results with the two-test versus three-test algorithms. Finally, the concordance between the VCT and laboratory rapid test results for positive/negative and strong/weak classification was estimated using Cohen's $\kappa$ coefficient.

### Results

Of 336 participants recruited for the survey, 55 were excluded because they were less than 18 years old ($n = 12$); considered noncompliant ($n = 2$); and previously diagnosed HIV-positive ($n = 41$).

Therefore, a total of 281 samples, 118 (42.0%) from Kisangani and 163 (58.0%) from Bunia, were analysed. **Figure 1** presents the number and proportions of positive and negative results of each HIV serological tests.

We found 100% concordance ($\kappa = 1$) between the VCT and laboratory rapid test results for the first rapid test (Alere Determine™ HIV-1/2), and 99.6%

concordance ($\kappa = 0.95$) for the second rapid test (Uni-Gold™ HIV). Taking into account the two-test algorithm in VCT, 15 samples were positives in the first test, but only 12 samples were confirmed positives in the second test. Three samples (1.1%; 95% CI: 0.4 – 3.1) were inconclusive.

However, taking into account the three-test algorithm in reference laboratory, 15 sera were positive in the first test, 13 sera were positive in the second test (12 strongly positive and one weakly positive). One serum was indeterminate in the third confirmatory test, reducing the rate of inconclusive test to 0.4% (95% CI: 0.1 – 2.1). The difference between the inconclusive results of the two-test versus three-test algorithms was statically significant ($P < 0.001$). One in five (20%) HIV-positive samples with first test were inconclusive with the two-test algorithm, whereas one in 15 (6.7%) samples with the three-test algorithm. Finally, 12 sera were concluded HIV positive, giving the overall prevalence of HIV infection to 4.3% (95% CI: 2.5 – 7.4). In addition, two of 12 HIV-positive sera (16.7%; 95% CI: 4.7 – 44.8) were co-infected by HIV-1 and HIV-2.

### Discussion

Our findings demonstrate that the prevalence of inconclusive results based on WHO II algorithm for HIV testing was higher (1.1%) than the 2012-revised and 2015-consolidated WHO strategy (0.4%). While, 20% of HIV-positive samples with first test were inconclusive with the two-test algorithm. These findings strongly support the need for confirmation testing of inconclusive HIV test results. Several studies have reported high prevalence of inconclusive sera in Central African countries. However, the frequency of inconclusive sera varies depending on the setting, ranging from 3.4% in the Central African Republic [9] and 8.4% to 9.0% in Cameroon [10, 11]. Nevertheless, the prevalence of inconclusive sera appears to be lower in West Africa, as reported in Burkina Faso (1.3%) [12], high

prevalence of inconclusive sera have also been reported in East Africa, with a prevalence of 12.2% in Tanzania [13]. In addition, a previous study conducted by Klarkow ski et al. in Bukavu, SouthKivu province of DRC, found a high rate of false positive results compared to Western Blot with twotest algorithm [14].

The etiology of indeterminate HIV serological reactivity for Central Africa people remains unknown. Reported causes of indeterminate or discrepant HIV test results are multifactorial and include early HIV infection; false positive reactions

autoimmu nity, pregnancy and vaccinations for influenza, hepatitis B, and rabies [2, 3]. In addition, false positive results have been associated with infectious diseases such as Epstein-Barr infection, uncomplicated malaria, sleeping sickness due to *Trypanosoma brucei gambiense*, schistosomia sis, leishmaniasis, syphilis and dengue [3, 15-17]. These infectious diseases are associated with immune stimulation with polyclonal B-cell activation, hyper-gammaglobuline mia and formation of circulating immune complexes. Circulating natural polyreactive antibodies, which are likely genetically controlled, can recognize HIV-1 antigens

Figure 1
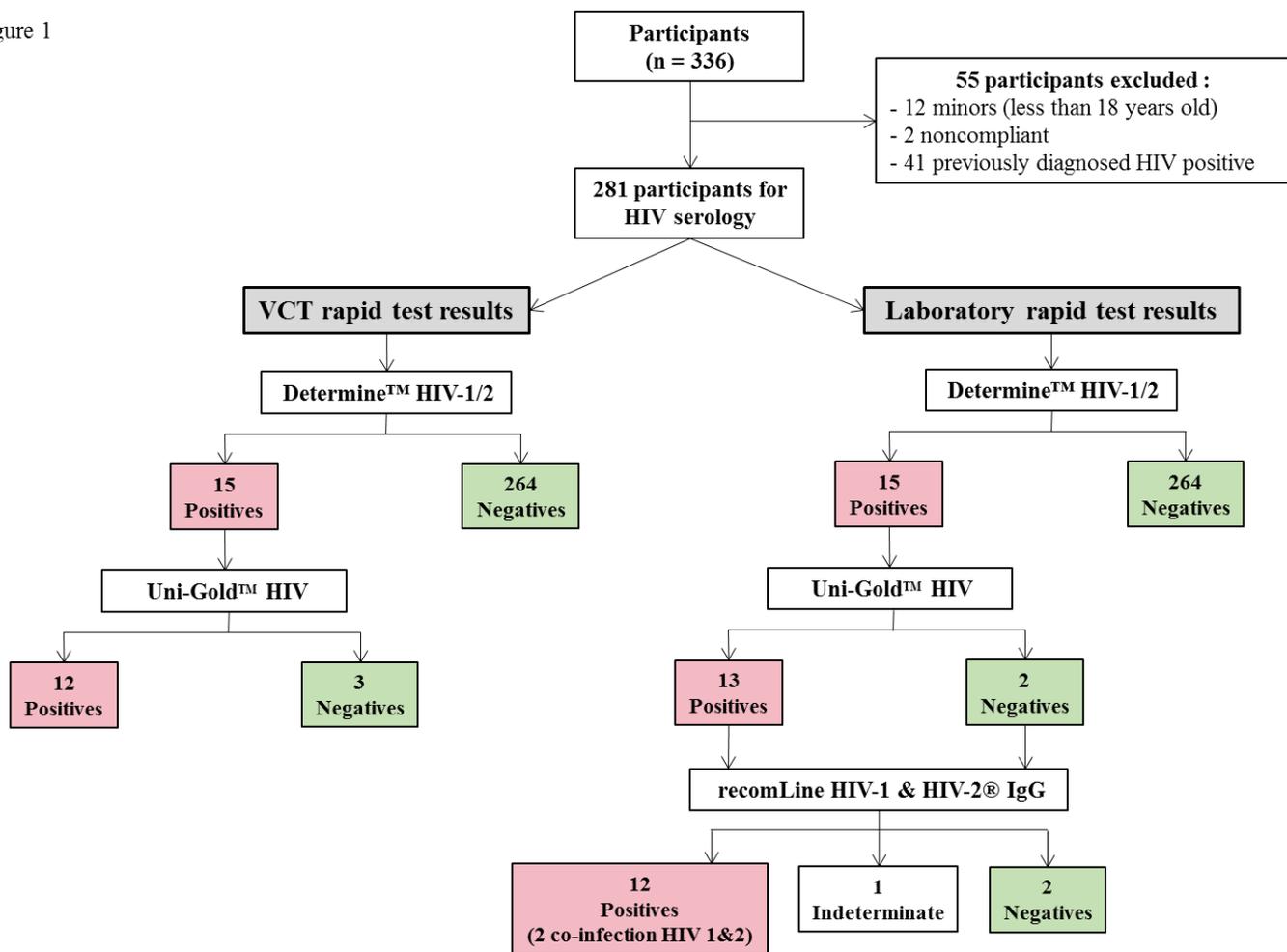


**Figure 1.** Flow chart showing the results of HIV testing by two-test algorithm proposed by the WHO in 1997 (WHO II) at the five voluntary and counselling testing sites versus the three-test algorithm according to the 2012-revised and

due to various conditions associated with 2015-consolidated WH

and contribute to indeterminate or equivocal results [3]. Moreover, the implication for the choice of HIV tests in

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the 2015 consolidated WHO HIV testing strategy appears to have another factor that increases the rate of inconclusive results in Central Africa [18].

In our series, the prevalence of HIV-1 and HIV-2 coinfection is high (16.7%). Few data in the literature corroborate this high rate of HIV-1 and HIV-2 co-infection in the context of Central Africa. Whereas antiretroviral therapy is different in HIV-1 mono-infected patients versus those co-infected with HIV-1 and HIV-2, the diagnosis of HIV-1 and HIV-2 discrimination is *de facto* necessary. This would facilitate the therapeutic continuum in a context where HIV-2 infection is poorly documented.

**Conclusion**

Our observations show that the HIV testing strategy by the three-test algorithm as per WHO [7, 8] significantly

Inconclusive results with HIV serodiagnosis algorithms,... reduces the rate of inconclusive results. These observations are very remarkable in a context where the rate of false positives is high because of the several endemic diseases responsible for cross-reactions. Our observations also show that the prevalence of HIV-1 and HIV-2 co-infection is high. Therefore, a large-scale research is essential to clarify these results.

### References

1. World Health Organization. WHO recommendations to assure HIV testing quality. Policy brief. Available at: http://www.who.int/hiv/pub/toolkits/policy-hiv-testingquality-assurance/en/ (Last accessed December 20, 2017).
   Google Scholar

2. Klarkowski D, O'Brien DP, Shanks L, Singh KP. Causes of false-positive HIV rapid diagnostic test results. Expert Rev Anti Infect Ther. 2014; 12: 49-62. **PubMed | Google Scholar | CrossRef**

3. Mbopi-Keou FX, Ndjoyi-Mbiguino A, Talla F, et al. Association of inconclusive sera for human immunodeficiency virus infection with malaria and EpsteinBarr virus infection in Central Africa. J Clin Microbiol. 2014; 52: 660-662. PubMed | Google Scholar | CrossRef

4. RDC. Ministère du Plan et Suivi de la Mise en oeuvre de la Révolution de la Modernité (MPSMRM), Ministère de la Santé Publique (MSP) et ICF International, 2014. Enquête Démographique et de Santé en République Démocratique du Congo 2013-2014. Rockville, Maryland, USA : MPSMRM,

MSP et ICF International. Available at: http://dhsprogram.com/FR300.pdf.2014. (last accessed 29 March 2017). Google Scholar

5. Tonen-Wolyec S, Batina-Agasa S, Muwonga J, Fwamba N'kulu F, Mboumba Bouassa RS, Bélec L. Evaluation of the practicability and virological performance of finger-stick whole-blood HIV self-testing in French-speaking subSaharan Africa. PLoS ONE. 2018; 13(1): e0189475. PubMed | Google Scholar | CrossRef

6. World Health Organization (1997) WHO recommendations for HIV antibody testing Wkly Epidemiol Rec 1997; 72: 81-88. PubMed | Google Scholar

7. World Health Organization (2012) Service delivery approaches to HIV testing and counselling: A strategic policy framework. Available at: http://apps.who.int/iris/bitstream/10665/75206/1/97892 41593877_eng.pdf (last access September 20, 2017). Google Scholar

8. World Health Organization. Consolidated guidelines on HIV testing services. Geneva: World Health Organization. July 2015. Available at: http://www.who.int/hiv/pub/guidelines/hiv-testingservices/en/ (last accessed July 27, 2017). Google Scholar

9. Gresenguet G, Sehonou J, Bassirou B, et al. Voluntary HIV counseling and testing: experience among the sexually active population in Bangui, Central African Republic. J Acquir Immune Defic Syndr. 2002; 31: 106-114. PubMed | Google Scholar

10. Aghokeng AF, Ewane L, Awazi B, et al. Evaluation of four simple/rapid assays and two fourth-generation ELISAs for the identification of HIV infection on a serum panel representing the HIV-1 group M genetic diversity in Cameroon. J Acquir Immune Defic Syndr. 2004; 37: 1632-1640. PubMed | Google | Scholar

11. Aghokeng AF, Mpoudi-Ngole E, Dimodi H, et al. 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. PubMed | Google Scholar | CrossRef

12. Kania D, Fao P, Valea D, et al. Low prevalence rate of indeterminate serological human immunodeficiency virus results among pregnant women from Burkina Faso, West Africa. J Clin Microbiol. 2010; 48: 1333-1336. PubMed | Google Scholar | CrossRef

13. Lyamuya EF, Aboud S, Urassa WK, et al. Evaluation of simple rapid HIV assays and development of national rapid

HIV test algorithms in Dar es Salaam, Tanzania. BMC Infect Dis. 2009; 9: 19.  PubMed | Google Scholar | CrossRef

14. Klarkowski DB, Wazome JM, Lokuge KM, et al. The evaluation of a rapid in situ HIV confirmation test in a

Inconclusive results with HIV serodiagnosis algorithms,…                                       Tonen-Wolyes S  et al.

programme with a high failure rate of the WHO HIV twotest diagnostic algorithm. PLoS One. 2009; 4: e4351.  PubMed | Google Scholar | CrossRef

15. Watt G, Chanbancherd P, Brown AE. Human immunodeficiency virus type 1 test results in patients with malaria and dengue infections. Clin Infect Dis. 2000; 30: 819.  PubMed | Google Scholar | CrossRef

16. Gasasira AF, Dorsey G, Kamya MR, et al. False-positive results of enzyme immunoassays for human immunodeficiency virus in patients with uncomplicated malaria. J Clin Microbiol. 2006; 44: 3021-3024.  PubMed | Google Scholar | CrossRef

17. Lejon V, Ngoyi DM, Ilunga M, et al. Low specificities of HIV diagnostic tests caused by Trypanosoma brucei gambiense sleeping sickness. J Clin Microbiol. 2010; 48: 2836-2839.  PubMed | Google Scholar | CrossRef

18. Jenabian M-A, Costiniuk CT, Talla P, Robin L, Tonen Wolyec S, Mboumba Bouassa RS, et al. Potential for FalsePositive Results with Serological Assays for HIV in Central Africa: Implications for the HIV Serodiagnosis Algorithm According to the 2015 Consolidated WHO Recommendations for Resource-Constrained Countries. AIDS Res Hum Retroviruses. 2017; 33(11):1077–1079.
   PubMed | Google Scholar | CrossRef