Are confirmatory assays reliable for HIV-1 / HIV-2 infection differentiation? A multicenter study

Vincent Guiraud, Jonathan Bocobza, Marion Desmonet, Florence Damond, Jean-Christophe Plantier, Ghislaine Moreau, Marc Wirden, Karl Stefic, Francis Barin, Agnès Gautheret- Dejean

Are confirmatory assays reliable for HIV-1 / HIV-2 infection differentiation? A multicenter study

Vincent Guiraud,[&a,b] Jonathan Bocobza,[&a] Marion Desmonet,[a] Florence Damond,[c,d] Jean-Christophe Plantier,[e] Ghislaine Moreau,[a] Marc Wirden,[a,b] Karl Stefic,[f] Francis Barin,[f] Agnès Gautheret-Dejean[*a,g]

Affiliations:

[a]AP-HP, Hôpitaux Universitaires La Pitié Salpêtrière-Charles Foix, Service de Virologie, F-75013 Paris, France

[b]Sorbonne Université, INSERM U1136, Institut Pierre Louis d'Epidémiologie et de Santé Publique (IPLESP)

[c]AP-HP, University Hospital Bichat-Claude Bernard, Service de Virologie, F-75018 Paris, France

[d]Université Paris cité, IAME, INSERM, F-75018 Paris, France

[e]Université de Rouen Normandie, Inserm UMR1311 DYNAMICURE, et CHU de Rouen, Laboratoire de virologie associé au CNR du VIH, F-76000 Rouen, France

[f]Université de Tours, UMR Inserm 1259, and CHU de Tours, Laboratoire associé au CNR du VIH, F-37000 Tours, France

[g]Université Paris cité, INSERM UMR-S 1139, 3PHM, F-75006 Paris, France

* Corresponding author, E-mail: agnes.gautheret@aphp.fr

& These authors contributed equally to this work.

ABSTRACT

Immunoblots remain the gold standards for HIV-1/HIV-2 infection confirmation. However, their ability to differentiate HIV-1 from HIV-2 infection on an antigenically diversified HIV-1 and HIV-2 panel remain scarce. We performed a multicenter study on 116 serum samples accounting for most HIV-1 (9 different subtypes in group M, 17 CRFs, 3 group O) and HIV-2 (groups A and B) diversity, evaluating seven confirmatory assays (six commercially available assays and one in-house assay) with genotyping as reference. The assays were INNO-LIA HIV I/II Score, HIV-2 Blot 1.2, HIV Blot 2.2, New Lav Blot I and II, Geenius and an in-house Serotyping ELISA. Among HIV-1 samples, INNO-LIA, HIV Blot 2.2, New Lav Blot I, Geenius and Serotyping had comparable high sensitivities, from 98% to 100%, whereas HIV-2 Blot 1.2 and New Lav Blot II had a high undetermined rate (85% and 95%, respectively). HIV-2 Blot 1.2 and New Lav Blot II misclassified 7% and 5% HIV-1 samples as HIV-2, respectively, and HIV-2 Blot 1.2 had a 8% false-negative rate. Among HIV-2 samples, INNO-LIA, New Lav Blot II, HIV-2 Blot 1.2 and Serotyping had high sensitivities, from 96% to 100%. HIV Blot 2.2 misclassified 17% HIV-2 samples as HIV-1/HIV-2 dual infections. New Lav Blot I misclassified 19% of HIV-2 samples as HIV-1 with a high (81%) undetermined rate, and Geenius misclassified 2% as HIV-1 and 7% as untypable HIV-positive. For HIV-1/HIV-2 dual infection, results were less sensitive with at most 87.5% for INNO-LIA and Geenius, and 75% for HIV Blot 2.2 and Serotyping. Overall, confirmatory assays remain useful for most cases, with the exception of HIV-1/HIV-2 dual infection suspicion.

KEYWORDS HIV confirmation, Western blot, INNO-LIA, Geenius, HIV-1, HIV-2, differentiation

Fundings

This work received no specific funding

Declaration of Competing Interests

The authors declare that they have no competing financial interests or personal relationships that could have influenced the work reported in this paper.

Acknowledgments

The authors thanks ADEBIOPHARM ER28 association for the financial participation which allowed us to present our results in congresses. The authors thank Bio-Rad, MP Biomedicals and Innogenetics

for providing free kits for this study. Laboratories that gave HIV detection kits had no part on study design, data collection, data analyses, data interpretation nor manuscript writing.

According to the World Health Organization data, around 38 million people are living with HIV-1 worldwide, of whom 26 million in Africa (1). HIV-2 is much less prevalent, with an estimated 2 million people infected (2), mainly in West African countries with prevalence up to 2.8 % (3–5). HIV-2 is infrequent in other parts of the world, accounting for new HIV cases from 0.1 % in the United States to 2% in France and 5 % in Portugal (6–8). Misclassifying HIV-2 can have deleterious consequences for the follow-up of infection since all HIV-1 viral load commercial assays fail to detect HIV-2 (9), and for antiretroviral therapy due to a natural resistance to all non-nucleoside reverse transcriptase and fusion inhibitors (10), and a lower efficacy of both some protease inhibitors (11) and the newly developed capsid inhibitor lenacapavir (12). For instance, the recent analysis of a cohort of Guinean pregnant women noticed that three quarters of infants from HIV-2 positive mothers received ineffective antiretroviral therapy at birth (4) due to the use of Nevirapine, a non-nucleoside inhibitor, as a first line treatment for newborns (13). Diagnosis of HIV-1 or HIV-2 infection is based on the detection of antibodies to HIV by enzyme immunoassays (ELISA) or rapid lateral flow assays followed by a specific confirmatory assay, or combination of rapid assays, which may differentiate HIV-1 from HIV-2 (14). Western blot have been the historical gold standard for differentiation (15), but only few studies have compared them to the more accurate molecular HIV-1 and HIV-2 RNA/DNA genotyping.

The objective of this study was to assess the accuracy of seven confirmatory assays to discriminate between an infection by either HIV-1 or HIV-2, or a dual infection (HIV-1/HIV-2), using a large panel of well characterized serum or plasma samples accounting for a large part of HIV-1 and HIV-2 diversities.

MATERIALS AND METHODS

Study population. Samples were originated from the routine of the lab. They corresponded to chronically untreated HIV-infected patients from four French University Hospitals (Bichat-Paris, Pitié Salpêtrière-Paris, Rouen and Tours) were included. Selection of HIV-1, HIV-2 or HIV-1/HIV-2 infections were assessed respectively by the detection of HIV-1 RNA (Cobas® AmpliPrep/Cobas TaqMan® HIV-1 Test, v2.0; Roche Diagnostics, Manheim Germany, or Real-Time HIV-1, Abbott Molecular, Rungis France) or HIV-2 RNA as previously described (16). In case of undetectable plasma viral load, typing was performed by quantification of HIV-1 proviral DNA (Biocentric generic DNA cell

for HIV-1, Biocentric, Bandol France) or HIV-2 DNA by an in-house method (17). Subtypes and recombinant forms of HIV-1 strains were determined using nucleotide sequences of the protease and reverse transcriptase genes and a Basic Local Alignment Search Tool (18) with the Los Alamos laboratory HIV-1 references , except for HIV-1 group O or HIV-2 for which in-house methods were used (19, 20). Serum samples were stored frozen at -20°C. Each sample was aliquoted to avoid several freeze-thawing cycles and was tested within the same thawing cycle for each assay.

Assays evaluated. Assays tested were INNO-LIA HIVI/II Score (Innogenetics, Gent, Belgium), HIV-2 Blot 1.2 and HIV Blot 2.2 (MP Biomedicals, Illkirch, France), New Lav Blot I, New Lav Blot II and Geenius (Bio-Rad laboratories, Marnes-la-Coquette, France), and a Serotyping ELISA (SS-ELISA), an in-house method used by the French National Reference Center for HIV-1 Serotyping (21). Each assay is described in Table 1. Commercial assays were performed and interpreted according to the manufacturers' recommendations. For INNO-LIA and Geenius, samples reactive for both HIV-1 and HIV-2 specific antibodies are classified as "positive for HIV antibodies (untypable)". As previously described for INNO-LIA (22), to ease readability in this paper, we termed this category as "HIV-1/HIV-2 dual reactive". Sensitivities for HIV-1, HIV-2 and HIV-1/HIV-2 dual infection were defined as the ability to identify respectively HIV-1, HIV-2 or HIV-1/HIV-2 dual infection. All confirmatory assays were prospectively performed at the Pitié Salpetrière Hospital except for Serotyping, which was prospectively performed in Tours University Hospital. Reading and assessment of HIV-1, HIV-2 and HIV-1/HIV-2 infections were done by 2 blinded operators. Results from Geenius and INNO-LIA were assessed using their respective readers.

Statistical analysis. Statistical analysis was conducted using R version 4.2.1 software (23). The 95% confidence intervals (95% CI) were calculated using Wilson confidence interval for proportions (24). We determined a priori that considering a 95% sensitivity for each test, with 61 samples (for HIV-1) we would have about 7% accuracy, and with 47 samples (for HIV-2) about 8% accuracy. We considered that these precisions would be enough to be clinically relevant.

Ethics. This work was approved by the French Infectious Disease Research Ethics Board (IRB00011642), no. 2023-0108. Patients samples were anonymized prior to the study in accordance with local ethics guidelines.

RESULTS

Samples panels. In this study, 116 samples were gathered from patients with chronic HIV infections: 61 samples from people infected with HIV-1 (except 47 for Geenius), 47 samples from people infected with HIV-2 (except 45 for Geenius), and 8 samples from those with HIV-1/HIV-2 dual infection. Subtypes and recombinant forms (genotypes) of HIV-1 strain accounted for most of its

antigenic diversity within HIV-1 group M: subtypes A (n=5), B (n=8), C (n=5), D (n=3), F (n=2), G (n=2), H (n=2), J (n=2), K (n=2) and Circulating Recombinant Forms (CRF) 05/06/11/12/13/14/15/18/19/22/27/30/36/42 (1 each), CRF01 and CRF09 (n=2 each), and CRF02 (n=9). We used three serum samples from HIV-1 group O infected patients. Of the 47 HIV-2 infected patients, seven were related to the group A and 13 to the group B, while 27 remained undetermined.

Accuracies for HIV-1 infection only. Assays designed to confirm HIV-1 infection specifically (INNO-LIA, HIV Blot 2.2, New Lav Blot I, Geenius and Serotyping) had similar high sensitivities (98% to 100%) with a low undetermined rate and no misclassification (Table 2). Of note, the only sample with undetermined result for HIV-Blot 2.2 corresponded to one of the three HIV-1 group O samples. Otherwise, tests designed to confirm HIV-2 infection only (HIV-2 Blot 1.2 and New Lav Blot II) exhibited very high rate of undetermined results (85% to 95%). This phenomenon is linked to cross-reactivities of HIV-1 antibodies toward HIV-2 GAG (mostly p26 for HIV-2 Blot 1.2 and New Lav Blot II), POL (34 and 66 for HIV-2 Blot 1.2 and New Lav Blot II), and ENV (gp105 for HIV-2 Blot 1.2 and New Lav Blot) specific antigens.

Accuracies for HIV-2 infection only. Assays designed to confirm HIV-2 infection specifically (HIV-2 Blot 1.2, New Lav Blot II and HIV Blot 2.2, INNO-LIA Geenius and serotyping) had HIV-2 sensitivities ranging from 83% to 100% (Table 3). INNO-LIA and Geenius classified respectively 2% (95% CI, 0.3% to 11%) and 7% (95% CI, 2% to 18%) of the samples as HIV-positive without differentiation. Geenius misclassified 2% (95% CI, 0.4% to 11%) samples as HIV-1 infection. New Lav Blot I misclassified 19 % (95% CI, 10% to 33%) samples as HIV-1 infection. HIV Blot 2.2 misclassified as possible HIV-1/HIV-2 dual infection 17% (95% CI, 8% to 30%) samples. These phenomena are due to cross-reactivities of HIV-2 antibodies toward HIV-1 GAG (p24, p40, and p55 proteins for New Lav Blot I, p24 and p40 for HIV Blot 2.2), POL (p34 and p68 proteins for New Lav Blot I, p34 for HIV Blot 2.2) specific antigens.

Accuracies for HIV-1/HIV-2 dual infection. Only four methods, INNO-LIA, HIV Blot 2.2, Geenius and Serotyping, were designed to identify HIV-1/HIV-2 dual infections, due to the presence of specific proteins for both viruses. INNO-LIA and Geenius performed well, with 7 of 8 samples being HIV-1/HIV-2 dually reactive. HIV Blot 2.2 and Serotyping accurately identified 6 dual infections among 8 samples. HIV-2 Blot 1.2, New Lav Blot I and New Lav Blot II were positive for all samples for their corresponding HIV type, HIV-1 or HIV-2 (table 4).

DISCUSSION

This study aimed to evaluate the accuracy of seven HIV confirmatory assays to identify an infection with HIV-1, HIV-2, or HIV-1/HIV-2, on a panel of serum or plasma samples designed to account for most HIV-1 and HIV-2 diversity (25). To date, few studies have already addressed this issue, as most used Western blot or INNO-LIA as the gold standard, instead of genotypes identification (22, 26–34). This study analyzed samples from 9 HIV-1 group M subtypes and 17 different HIV-1 CRFs, HIV-1 group O, and at least 2 of the main HIV-2 groups (35).

Considering HIV-1 infection only, INNO-LIA, HIV Blot 2.2, New Lav Blot I, Geenius and Serotyping had similar sensitivities, from 98% to 100%. On the opposite, assays designed only to identify HIV-2 infection, HIV-2 Blot 1.2 and New Lav Blot II, exhibited high rates of undetermined results and misclassified as HIV-2 some HIV-1 only infections (7% and 5%, respectively), a consequence of cross-reactivity between HIV-1 antibodies and HIV-2 proteins. These results are consistent with previous studies regarding HIV-1 infection sensitivities and undetermined results for INNO-LIA (27, 36, 37), HIV Blot 2.2 (36, 38), New Lav Blot I and II (39, 40) and Geenius (27, 36–39, 41, 42), summarized in Fig. 1.

For HIV-2 infection only, sensitivities ranged from 91% to 100% with methods designed to confirm HIV-2 infection (INNO-LIA, HIV-2 Blot 1.2, New Lav Blot II, Geenius and serotyping). Overall, results were consistent with previous studies. New Lav Blot II and HIV-2 Blot 1.2 had reported sensitivities of at least 95% (32, 43). INNO-LIA had a reported sensitivity of 100% (36, 37), while sensitivity ranged from 85% to 100% for Geenius (27, 39–42, 44). However, most of these studies included a very limited number of patients. HIV Blot 2.2 had an intermediate sensitivity of 83%, which corresponded to the HIV-2 specific antigen positivity, an antigen designed by the manufacturer to alert for a possible HIV-2 infection. New Lav Blot I misclassified a high proportion of HIV-2 infection as HIV-1 infections (19%) and exhibited a particularly high undetermined rate (80%), a result in accordance with previous studies (39, 43). Thus it should not be used alone for differentiation but in combination with a HIV-2 specific lateral flow or confirmatory assay. When considering the infections with HIV-1 or HIV-2 only, a higher occurrence of cross-reactivity was noted when HIV-2 antibodies interacted with HIV-1 proteins, as opposed to HIV-1 antibodies binding to HIV-2 proteins. This phenomenon has been described by Damond et al for HIV-2 group B (43). Our results, along with an extensive comparison to previous studies that used genotyping as the gold standard are summarized Fig. 1.

For HIV-1/HIV-2 dual infections, INNO-LIA and Geenius reacted with both HIV-1 specific and HIV-2 specific antigens for 87% of the samples. However, no assay exhibited a perfect concordance with

genotyping as the gold standard for this scarce situation, a finding consistent with Tchounga et al who used both Immunocomb BiSpot and Serotyping as the reference assay (45). In the event of a suspected dual infection, we advise to consider a genetic assay to confirm the diagnosis.

We must consider that our study has some limitations. Firstly, since by design the study focused on samples selected from patients having either HIV-1, or HIV-2 or HIV-1/HIV-2 infection, we could not determine predictive positive and negative value of each test. Secondly, we have not been able to identify the HIV-2 group concerned in half samples, not allowing to see if a group exhibited more cross-reactivities toward HIV-1 than others, as it was already observed for HIV-2 group B (43) . Lastly, the number of HIV-1/HIV-2 dual infections was low, even in a context of a multicentric study, reflecting the scarcity of such cases in France (46). As a consequence, assay accuracies presented for HIV-1/HIV-2 infection should be considered with caution, and further studies should be conducted on this population.

Our study demonstrated that commercial assays designed to confirm specifically either HIV-1 (New Lav Blot I) or HIV-2 (New Lav Blot II, HIV-2 Blot 1.2) infection exhibited non negligible cross-reactivity with HIV-2 or HIV-1, respectively, leading to misclassification. They should not be used alone for differentiation. On the opposite, assays designed to detect both HIV-1 and HIV-2 (HIV-Blot 2.2, INNO-LIA, Geenius, serotyping) performed well for both infections, although most assays exhibited some cross-reactivity, especially for HIV-2 positive samples. Overall, unresolved differentiation should alert to HIV-2 or dual infection, requiring further investigation, but misclassification of HIV-1 or HIV-2 single infection should be exceptional. However, no assay aligns perfectly with genotyping as the gold standard, especially for dual infections.

In conclusion, confirmatory assays have been the historical gold standard for HIV-1 and HIV-2 differentiation, and they remain useful and reliable for HIV-1/HIV-2 differentiation for most cases, with the exception of HIV-1/HIV-2 dual infection suspicion. However, they remain time-consuming, with a relative high cost. Altogether, these factors raise the question of the most suitable gold standard for differentiating HIV-1 from HIV-2, especially in the suspicion of dual infection or in the realm of clinical trials and research.

REFERENCES

1. 2021. Geneva: Joint United Nations Programme on HIV/AIDS. UNAIDS.

2. Nicolás D, Ambrosioni J, Paredes R, Marcos MÁ, Manzardo C, Moreno A, Miró JM. 2015. Infection with human retroviruses other than HIV-1: HIV-2, HTLV-1, HTLV-2, HTLV-3 and HTLV-4. Expert Review of Anti-infective Therapy 13:947–963.

3. Olesen JS, Jespersen S, da Silva ZJ, Rodrigues A, Erikstrup C, Aaby P, Wejse C, Hønge BL. 2018. HIV-2 continues to decrease, whereas HIV-1 is stabilizing in Guinea-Bissau. AIDS 32:1193–1198.

4. Rasmussen DN, Vieira N, Hønge BL, da Silva Té D, Jespersen S, Bjerregaard-Andersen M, Oliveira I, Furtado A, Gomes MA, Sodemann M, Wejse C, Unger HW. 2020. HIV-1 and HIV-2 prevalence, risk factors and birth outcomes among pregnant women in Bissau, Guinea-Bissau: a retrospective cross-sectional hospital study. Sci Rep 10:12174.

5. Wang C, Hawes SE, Gaye A, Sow PS, Ndoye I, Manhart LE, Wald A, Critchlow CW, Kiviat NB. 2007. HIV prevalence, previous HIV testing, and condom use with clients and regular partners among Senegalese commercial sex workers. Sexually Transmitted Infections 83:534–540.

6. Peruski AH, Wesolowski LG, Delaney KP, Chavez PR, Owen SM, Granade TC, Sullivan V, Switzer WM, Dong X, Brooks JT, Joyce MP. 2020. Trends in HIV-2 Diagnoses and Use of the HIV-1/HIV-2 Differentiation Test — United States, 2010–2017. MMWR Morb Mortal Wkly Rep 69:63–66.

7. Carvalho A, Valadas E, França L, Carvalho C, Aleixo M, Mendez J, Marques R, Sarmento A, Doroana M, Antunes F, Branco T, Águas M, Sarmento e Castro R, Lazarus J, Barros H. 2012. Population mobility and the changing epidemics of HIV-2 in Portugal: Changing HIV-2 epidemics in Portugal. HIV Med n/a-n/a.

8. Barin F, Cazein F, Lot F, Pillonel J, Brunet S, Thierry D, Damond F, Brun-Vézinet F, Desenclos J-C, Semaille C. 2007. Prevalence of HIV-2 and HIV-1 group O infections among new HIV diagnoses in France: 2003–2006. AIDS 21:2351–2353.

9. Parekh BS, Ou C-Y, Fonjungo PN, Kalou MB, Rottinghaus E, Puren A, Alexander H, Hurlston Cox M, Nkengasong JN. 2018. Diagnosis of Human Immunodeficiency Virus Infection. Clin Microbiol Rev 32:e00064-18.

10. Reeves I, Cromarty B, Deayton J, Dhairyawan R, Kidd M, Taylor C, Thornhill J, Tickell-Painter M, van Halsema C. 2021. British HIV Association guidelines for the management of HIV-2 2021. HIV Medicine 22:1–29.

11. Mahdi M, Szojka Z, Mótyán J, Tőzsér J. 2015. Inhibition Profiling of Retroviral Protease Inhibitors Using an HIV-2 Modular System. Viruses 7:6152–6162.

12. Smith R, Raugi D, Nixon R, Seydi M, Margot N, Callebaut C, Gottlieb GS. 2023. Antiviral Activity of Lenacapavir Against HIV-2 Isolates. Poster. CROI - Conference on Retroviruses and Opportunistic Infections.

13. Ryom L, De Miguel R, Cotter AG, Podlekareva D, Beguelin C, Waalewijn H, Arribas JR, Mallon PWG, Marzolini C, Kirk O, Bamford A, Rauch A, Molina JM, Kowalska JD, Guaraldi G, Winston A, Boesecke C, Cinque P, Welch S, Collins S, Behrens GMN, the EACS Governing Board. 2022. Major revision version 11.0 of the European AIDS Clinical Society Guidelines 2021. HIV Medicine 23:849–858.

14. World Health Organization. 2020. Consolidated guidelines on HIV testing services, 2019. World Health Organization, Geneva. https://apps.who.int/iris/handle/10665/336323. Retrieved 28 November 2022.

15. Nishanian P, Taylor JM, Korns E, Detels R, Saah A, Fahey JL. 1987. Significance of quantitative enzyme-linked immunosorbent assay (ELISA) results in evaluation of three ELISAs and Western blot tests for detection of antibodies to human immunodeficiency virus in a high-risk population. J Clin Microbiol 25:395–400.

16. Damond F, Collin G, Descamps D, Matheron S, Pueyo S, Taieb A, Campa P, Benard A, Chêne G, Brun-Vezinet F. 2005. Improved Sensitivity of Human Immunodeficiency Virus Type 2 Subtype B Plasma Viral Load Assay. J Clin Microbiol 43:4234–4236.

17. Damond F, Loussert-Ajaka I, Apetrei C, Descamps D, Souquière S, Leprêtre A, Matheron S, Brun-Vézinet F, Simon F. 1998. Highly Sensitive Method for Amplification of Human Immunodeficiency Virus Type 2 DNA. J Clin Microbiol 36:809–811.

18. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. Journal of Molecular Biology 215:403–410.

19. Janssens W, Fransen K, Loussert-Ajaka I, Heyndrickx L, Ivens T, Eberle J, Nkengasong J. 1995. Diagnosis of HIV-1 group O infection by polymerase chain reaction. The Lancet 346:451–452.

20. Damond F, Brun-Vezinet F, Matheron S, Peytavin G, Campa P, Pueyo S, Mammano F, Lastere S, Farfara I, Simon F, Chene G, Descamps D. 2005. Polymorphism of the Human Immunodeficiency Virus Type 2 (HIV-2) Protease Gene and Selection of Drug Resistance Mutations in HIV-2-Infected Patients Treated with Protease Inhibitors. J Clin Microbiol 43:484–487.

21. Barin F, Plantier J-C, Brand D, Brunet S, Moreau A, Liandier B, Thierry D, Cazein F, Lot F, Semaille C, Desenclos J-C. 2006. Human immunodeficiency virus serotyping on dried serum spots as a screening tool for the surveillance of the AIDS epidemic. J Med Virol 78:S13–S18.

22. Hønge B, Jespersen S, Medina C, Té D, da Silva Z, Christiansen M, Kjerulff B, Laursen A, Wejse C, Krarup H, Erikstrup C, the Bissau HIV Cohort study group. 2018. The challenge of discriminating between HIV-1, HIV-2 and HIV-1/2 dual infections. HIV Med 19:403–410.

23. R Core Team. R: A Language and Environment for Statistical Computing.

24. Newcombe RG. 1998. Two-sided confidence intervals for the single proportion: comparison of seven methods. Statist Med 17:857–872.

25. Bbosa N, Kaleebu P, Ssemwanga D. 2019. HIV subtype diversity worldwide. Current Opinion in HIV and AIDS 14:153–160.

26. Serhir B, Desjardins C, Doualla-Bell F, Simard M, Tremblay C, Longtin J. 2019. Evaluation of the Bio-Rad Geenius HIV 1/2 Assay as Part of a Confirmatory HIV Testing Strategy for Quebec, Canada: Comparison with Western Blot and Inno-Lia Assays. J Clin Microbiol 57:e01398-18.

27. Herssens N, Beelaert G, Fransen K. 2014. Discriminatory capacity between HIV-1 and HIV-2 of the new rapid confirmation assay Geenius. Journal of Virological Methods 208:11–15.

28. Hønge BL, Bjarnason Obinah MP, Jespersen S, Medina C, da Silva Té D, José da Silva Z, Østergaard L, Laursen AL, Wejse C, Erikstrup C. 2014. Performance of 3 Rapid Tests for Discrimination Between HIV-1 and HIV-2 in Guinea-Bissau, West Africa. JAIDS Journal of Acquired Immune Deficiency Syndromes 65:87–90.

29. Nkengasong JN, Maurice C, Koblavi S, Kalou M, Bile C, Yavo D, Boateng E, Wiktor SZ, Greenberg AE. 1998. Field Evaluation of a Combination of Monospecific Enzyme-Linked Immunosorbent Assays for Type-Specific Diagnosis of Human Immunodeficiency Virus Type 1 (HIV-1) and HIV-2 Infections in HIV-Seropositive Persons in Abidjan, Ivory Coast. J Clin Microbiol 36:123–127.

30. Chaillet P, Tayler-Smith K, Zachariah R, Duclos N, Moctar D, Beelaert G, Fransen K. 2010. Evaluation of four rapid tests for diagnosis and differentiation of HIV-1 and HIV-2 infections in Guinea-Conakry, West Africa. Transactions of the Royal Society of Tropical Medicine and Hygiene 104:571–576.

31. Abbate I, Pergola C, Pisciotta M, Sciamanna R, Sias C, Orchi N, Libertone R, Ippolito G, Capobianchi MR. 2014. Evaluation in a clinical setting of the performances of a new rapid confirmatory assay for HIV1/2 serodiagnosis. Journal of Clinical Virology 61:166–169.

32. Qiu M, Liu X, Jiang Y, Nkengasong JN, Xing W, Pei L, Parekh BS. 2009. Current HIV-2 diagnostic strategy overestimates HIV-2 prevalence in China. J Med Virol 81:790–797.

33. Rouet F, Ekouevi DK, Inwoley A, Chaix M-L, Burgard M, Bequet L, Viho I, Leroy V, Simon F, Dabis F, Rouzioux C. 2004. Field Evaluation of a Rapid Human Immunodeficiency Virus (HIV) Serial Serologic Testing Algorithm for Diagnosis and Differentiation of HIV Type 1 (HIV-1), HIV-2, and Dual HIV-1-HIV-2 Infections in West African Pregnant Women. J Clin Microbiol 42:4147–4153.

34. Ciccaglione AR, Miceli M, Pisani G, Bruni R, Iudicone P, Costantino A, Equestre M, Tritarelli E, Marcantonio C, Tataseo P, Marazzi MC, Ceffa S, Paturzo G, Doro Altan AM, Magnano San Lio M, Mancinelli S, Ciccozzi M, Lo Presti A, Rezza G, Palombi L. 2010. Improving HIV-2 Detection by a Combination of Serological and Nucleic Acid Amplification Test Assays. J Clin Microbiol 48:2902–2908.

35. Visseaux B, Bertine M, Le Hingrat Q, Ferré V, Charpentier C, Collin F, Damond F, Matheron S, Hué S, Descamps D. 2021. HIV-2 diversity displays two clades within group A with distinct geographical distribution and evolution. Virus Evolution 7:veab024.

36. Wong CC, Lim SH, Tan CT, Lui SY, Lee YL, Chan KP. 2018. Performance of the HIV Blot 2.2, INNO-LIA HIV I/II Score, and Geenius HIV 1/2 Confirmatory Assay for use in HIV confirmation. PLoS ONE 13:e0199502.

37. Tinguely C, Schild-Spycher T, Bahador Z, Gowland P, Stolz M, Niederhauser C. 2014. Comparison of a conventional HIV 1/2 line immunoassay with a rapid confirmatory HIV 1/2 assay. Journal of Virological Methods 206:1–4.

38. Moon H-W, Huh HJ, Oh GY, Lee SG, Lee A, Yun Y-M, Hur M. 2015. Evaluation of the Bio-Rad Geenius HIV 1/2 Confirmation Assay as an Alternative to Western Blot in the Korean Population: A Multi-Center Study. PLoS ONE 10:e0139169.

39. Kondo M, Sudo K, Sano T, Kawahata T, Itoda I, Iwamuro S, Yoshimura Y, Tachikawa N, Kojima Y, Mori H, Fujiwara H, Hasegawa N, Kato S. 2018. Comparative evaluation of the Geenius HIV 1/2 Confirmatory Assay and the HIV-1 and HIV-2 Western blots in the Japanese population. PLoS ONE 13:e0198924.

40. Kusagawa S, Kawana-Tachikawa A, Matsubayashi K, Hoshi Y, Ishimaru K, Hamaguchi I. 2021. Evaluation of Geenius HIV-1/2 Confirmatory Assay for the confirmatory and differential diagnosis of HIV-1/HIV-2 in Japan and reliability of the Geenius Reader in the diagnosis of HIV-2. BMC Infect Dis 21:569.

41. Malloch L, Kadivar K, Putz J, Levett PN, Tang J, Hatchette TF, Kadkhoda K, Ng D, Ho J, Kim J. 2013. Comparative evaluation of the Bio-Rad Geenius HIV-1/2 Confirmatory Assay and the Bio-Rad Multispot HIV-1/2 Rapid Test as an alternative differentiation assay for CLSI M53 algorithm-I. Journal of Clinical Virology 58:e85–e91.

42. Fordan S, Bennett B, Lee M, Crowe S. 2017. Comparative performance of the Geenius™ HIV-1/HIV-2 supplemental test in Florida's public health testing population. Journal of Clinical Virology 91:79–83.

43. Damond F, Apetrei C, Robertson DL, Souquière S, Leprêtre A, Matheron S, Plantier J, Brun-Vézinet F, Simon F. 2001. Variability of Human Immunodeficiency Virus Type 2 (HIV-2) Infecting Patients Living in France. Virology 280:19–30.

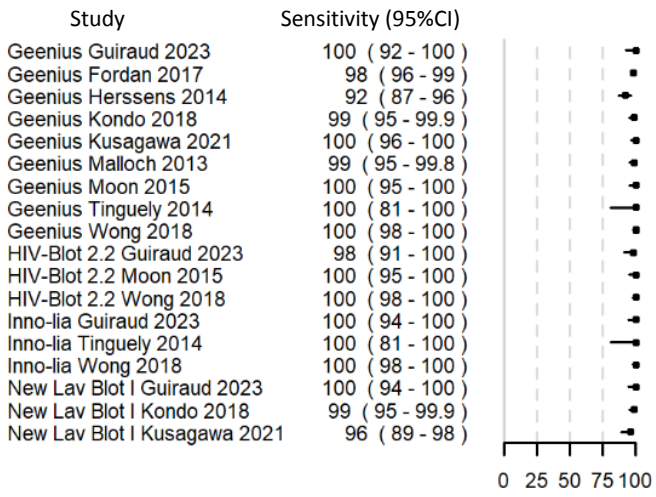
44. Luo W, Sullivan V, Smith T, Peters PJ, Gay C, Westheimer E, Cohen SE, Owen SM, Masciotra S. 2019. Performance evaluation of the Bio-Rad Geenius HIV 1/2 supplemental assay. Journal of Clinical Virology 111:24–28.

45. Tchounga BK, Inwoley A, Coffie PA, Minta D, Messou E, Bado G, Minga A, Hawerlander D, Kane C, Eholie SP, Dabis F, Ekouevi DK, for the WADA Collaboration. 2014. Re-testing and misclassification of HIV-2 and HIV-1&2 dually reactive patients among the HIV-2 cohort of The West African Database to evaluate AIDS collaboration. Journal of the International AIDS Society 17:19064.

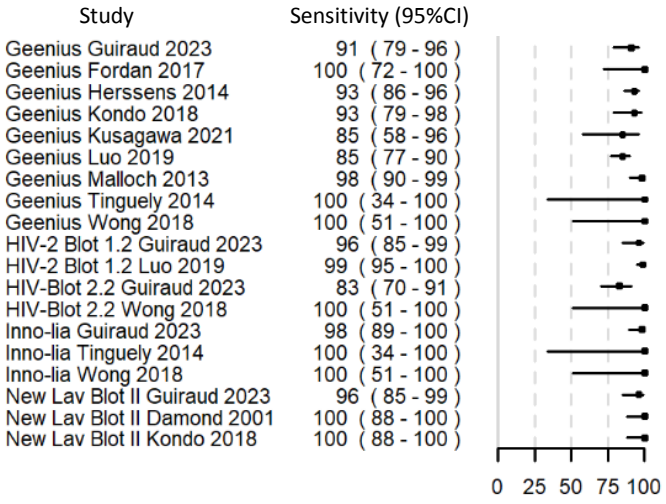
46. Cazein Françoise, Florence Lot, Josiane Pillonel, Yann le Strat, Cécile Sommen, Roselyne Pinget, Stéphane Le Vu, Sylvie Brunet, Damien Thierry, Denys Brand, Marlène Leclerc, Lofti Benyelles, Clara Da Costa, Francis Barin, Caroline Semaille. 2013. Dépistage du VIH et découvertes de séropositivité, France, 2003- 2010. Bulletin épidémiologique hebdomadaire. France.

47. Boobalan J, Torti A, Dinesha TR, Solomon SS, Balakrishnan P, Saravanan S. 2017. Cost-effective HIV-1 virological monitoring in resource-limited settings using a modified commercially available qPCR RNA assay. Journal of Virological Methods 248:71–76.

A. Sensitivities to detect HIV-1 infection



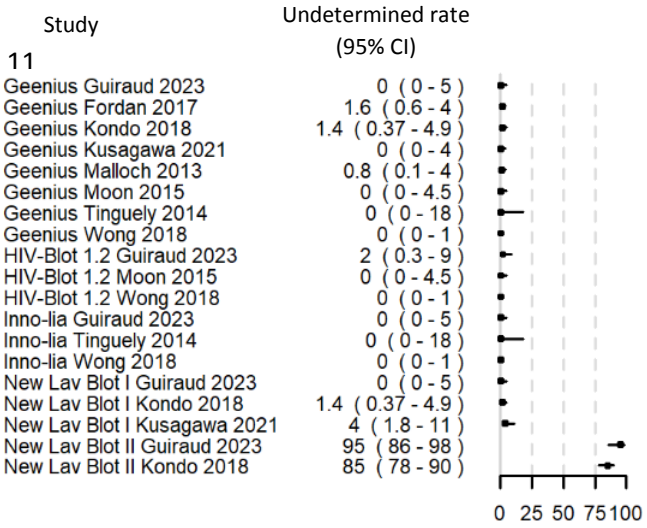
B. Sensitivities to detect HIV-2 infection



9

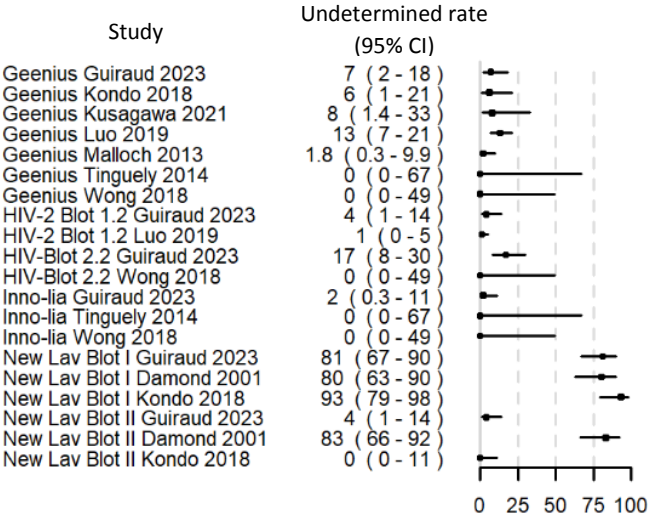
C. Undetermined rate to detect HIV-1 infection

10

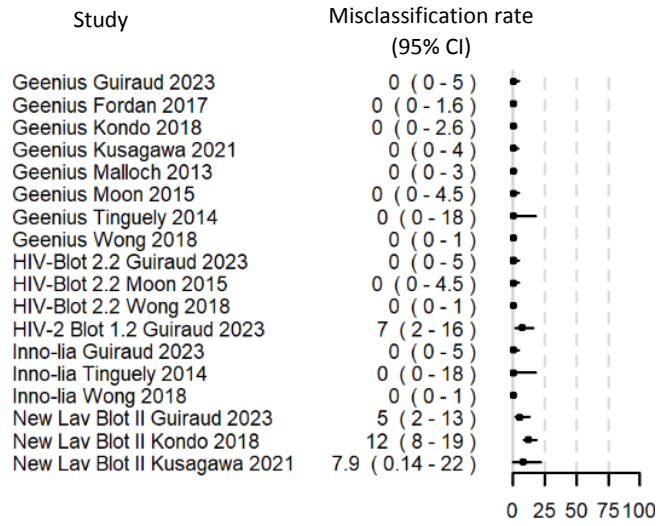


11

D. Undetermined rate to detect HIV-2 infection



E. Percentage of HIV-1 infection misclassified as HIV-2 infection



F. Percentage of HIV-2 infection misclassified as HIV-1 infection

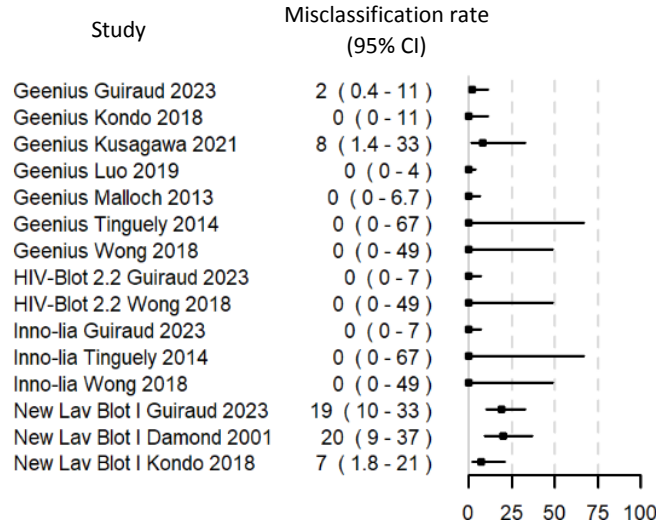


FIG 1 Comparison for the different tests among studies to accurately identify or misclassify HIV-1 or HIV-2 infection, with sensitivity for HIV-1 (A), HIV-2 (B) infection, undetermined rate for HIV-1 (C) and HIV-2 (D) samples, and percentage of HIV-1 samples misclassified as HIV-2 (E) or percentage of HIV-2 samples misclassified as HIV-1 (F).

TABLE 1 Description of kits and methods used

Kit / method evaluated	Antigens used	Technology	Matrix	Test duration	Cost per sample[c]
INNO-LIA HIV I/II Score	Sgp120[b], gp41[b], p31[a], p24[a], p17[a], gp36[b], and sgp105[b]	Line immunoblot	Serum, plasma	18h	11 US$
HIV Blot 2.2	HIV-1 viral lysate and peptide from HIV-2 envelope	Western blot	Serum, plasma	3h	28 US$
HIV-2 Blot 1.2	HIV-2 viral lysate	Western blot	Serum, plasma	3h	22 US$
New Lav Blot I	HIV-1 viral lysate	Western blot	Serum, plasma	4h	18 US$
New Lav Blot II	HIV-2 viral lysate	Western blot	Serum, plasma	4h	29 US$
Geenius	Gp160[a], gp41[b], p31[b], p24[a], gp140[b], and gp36[b]	Immunochromatography	Serum, plasma, whole blood	25 min	20-25 US$
Serotyping (SS-ELISA)	HIV-1 and HIV-2 peptides	ELISA	Serum, plasma	2h	1 US$

[a]Recombinant protein.

[b]Synthetic peptide.

[c]Reagents only.

	Methods						
HIV-1 positive panel *(n = 61)*	**INNO-LIA[a]**	**HIV-2 Blot 1.2[b]**	**HIV Blot 2.2**	**New Lav Blot I[c]**	**New Lav Blot II[b]**	**Geenius[a]**	**Serotyping (SS-ELISA)**
No. correctly identified/no. tested	61/61	NA	60/61	61/61	NA	47/47	61/61
Sensitivity to detect HIV-1 only % [95% CI][d]	100 [94-100]	NA	98 [91-100]	100 [94-100]	NA	100 [92-100]	100 [94-100]
No. misclassified as HIV-2 (%) [95% CI]	0 (0) [0-5]	4 (7) [2-16]	0 (0) [0-5]	NA	3 (5) [2-13]	0 (0) [0-5]	0 (0) [0-5]
No. false negative (%) [95% CI]	0 (0) [0-5]	5 (8) [3-18]	0 (0) [0-5]	0 (0) [0-5]	0 (0) [0-5]	0 (0) [0-5]	0 (0) [0-5]
No. undetermined (%) [95% CI]	0 (0) [0-5]	52 (85) [74-92]	1 (2) [0.3-9]	0 (0) [0-5]	58 (95) [86-98]	0 (0) [0-5]	0 (0) [0-5]

TABLE 2 Performance characteristics of INNO-LIA, HIV-2 Blot1.2, HIV Blot 2.2, New Lav Blot I, New Lav Blot II, Geenius and Serotyping methods for HIV-1 positive panel

[a]For INNOLIA and Geenius, results using an automatic reading are presented.

[b]HIV-2 Blot 1.2 and New Lav Blot II are designed to confirm only HIV-2 infection.

[c]New Lav Blot I is designed to confirm only HIV-1 infection.

[d]95% CI: Confidence Interval (95% CI).

	Methods						
HIV-2 positive panel *(n = 47)*	**INNO-LIA[a]**	**HIV-2 Blot 1.2[b]**	**HIV Blot 2.2**	**New Lav Blot I[c]**	**New Lav Blot II[b]**	**Geenius[a]**	**Serotyping (SS-ELISA)**
No. correctly identified/no. tested	46/47	45/47	39/47	NA	45/47	41/45	47/47
Sensitivity to detect HIV-2 [95% CI][d]	98 [89-100]	96 [85-99]	83[e] [70-91]	NA	96 [85-99]	91 [79-96]	100 [92-100]
No. misclassified as HIV-1 (%) [95% CI]	0 (0) [0-7]	NA	0 (0) [0-7]	9 (19) [10-33]	NA	1 (2) [0.4-11]	0 (0) [0-7]
No. misclassified as HIV positive (%) [95% CI][f]	1 (2) [0.3-11]	NA	8 (17) [8-30]	NA	NA	3 (7) [2-18]	0 (0) [0-7]

TABLE 3 Performance characteristics of INNO-LIA, HIV-2 Blot1.2, HIV Blot 2.2, New Lav Blot I, New Lav Blot II, Geenius and Serotyping methods for HIV-2 panel

No. undetermined (%)	0 (0)	2 (4)	0 (0)	38 (81)	2 (4)	0 (0)	0 (0)
[95% CI]	[0-7]	[1-14]	[0-7]	[67-90]	[1-14]	[0-7]	[0-7]

[a]For INNOLIA and Geenius, results using an automatic reading are presented.

[b]HIV-2 Blot 1.2 and New Lav Blot II are designed to confirm only HIV-2 infection.

[c]New Lav Blot I is designed to confirm only HIV-1 infection.

[d]95% CI: Confidence Interval (95% CI).

[e]At least positive for HIV-2 specific antigen

[f]HIV-1 and HIV-2 positive without differentiation.

TABLE 4 Performance characteristics of INNO-LIA, HIV-2 Blot1.2, HIV Blot 2.2, New Lav Blot I, New Lav Blot II, Geenius and Serotyping methods for dual HIV-1 and HIV-2 positive panel

HIV-1 and HIV-2 positive panel *(n = 8)*	Methods						
	INNO-LIA[a]	**HIV-2 Blot 1.2**[b]	**HIV Blot 2.2**	**New Lav Blot I**[c]	**New Lav Blot II**[b]	**Geenius**[a]	**Serotyping (SS-ELISA)**
No. correctly identified/no. tested	7/8[d]	NA	6/8[d]	NA	NA	7/8[d]	6/8
Sensitivity to detect HIV-1 and HIV-2 % [95% CI][e]	87.5 [53-98]	NA	75 [41-93]	NA	NA	87.5 [53-98]	75 [41-93]
No. misclassified as HIV-1 only (%) [95% CI]	0 (0) [0-32]	NA	1 (12.5) [2-47]	8 (100) [67-100]	NA	0 (0) [0-32]	1 (12.5) [2-47]
No. misclassified as HIV-2 only (%) [95% CI]	1 (12.5) [2-47]	8 (100) [67-100]	1 (12.5) [2-47]	NA	8 (100) [67-100]	1 (12.5) [2-47]	1 (12.5) [2-47]

[a]For INNOLIA and Geenius, results using an automatic reading are presented.
[b]HIV-2 Blot 1.2 and New Lav Blot II are designed to confirm only HIV-2 infection.
[c]New Lav Blot I is designed to confirm only HIV-1 infection.
[d]Reactive for both HIV-1 and HIV-2 specific antibodies.
[e]95% CI: Confidence Interval (95% CI).