# Fourth-Generation HIV Rapid Tests: Enhanced Sensitivity and Reduced Diagnostic Window for HIV-1 Primary Infection Screening

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**Fundings**

This work was supported in part by the ANRS-MIE (Agence Nationale de Recherches sur le SIDA et les hépatites virales-Maladies Infectieuses Emergentes) (Medical and Pharmacological ANRS-MIE Network), the Groupe Pasteur Mutualité and the Adebiopharm ER28 association

**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 Abbott and Prodiag for providing a part of the kits used in this study for free. Abbott and Prodiag had no part on study design, data collection, data analyses, data interpretation, manuscript writing nor decision to submit to publication.

**Word count:** Abstract 219, Text 2187

## Abstract

**Introduction:** As most HIV rapid tests (HRT) detect only HIV-1/2 antibodies, their performance during primary HIV infection is poor. Determine™ HIV Early detect (Abbott) (Determine) is the only HRT with a HIV-1 p24-antigen detection, but the impact of this addition in shortening diagnostic window remains unclear.

**Methods:** A total of 183 HIV-1 primary infection samples were tested using the HRTs Determine and ONE STEP anti-HIV (1&2) Test (InTec Products) (One-Step). The pre-seroconversion subgroup was defined as p24-antigen positivity without Western blot nor Liaison XL (4th generation enzyme immunoassaywith distinct signal for p24-antigen and HIV-1 antibody) HIV-1 antibodies.

**Results**: Global sensitivity (95%CI) was 95% (91-97) for Determine vs 80% (74-85%) for One-Step (difference p=1.38e-06). Pre-seroconversion subgroup sensitivity was lower, at 71.9 (54.6-84.4%) for Determine and 9.7% (3.3-24.9%) for One-Step. Among the 45 samples with an HIV-1 infection date, no HRT was reactive up to two weeks. Between two and three weeks, Determine sensitivity was 78% (45-95%) vs 56% (27-81%) for One-Step. From three weeks to one month Determine sensitivity was 90% (62-98%) and One-Step 45% (21-72%). The last negative sample occurred at three weeks for Determine vs 70-90 days for One-Step.

**Conclusion**: HRT with p24-antigen detection significantly shortens diagnostic window from approximatively three months to one month. HRTs should be used with caution in the first month after HIV infection.



**Keywords**: Determine Combo; One-Step; HIV primary infection; sensitivity; HIV rapid test; Diagnostic window

# Introduction

Since the release of the first FDA-approved HIV screening assay in 1985, four generations have been developed, each associated with a technical breakthrough [1]. The 1st generation was based on virus-infected cells. The second and third relied on recombinant antigens and synthetic peptides, improving both sensitivity and specificity [2]. Finally, the 4th generation introduced the detection of HIV-1 p24-antigen, which shortened the diagnostic window from 3-6 weeks to around 2 weeks post-infection [3–7].

As most HIV rapid tests (HRTs) are considered 3rd generation assays, they perform poorly in a primary HIV-1 infection setting, with sensitivities as low as 70% [8,9]. Determine™ HIV Early detect (Determine), the first FDA-approved 4th generation HRT, was designed to address this issue, with the independent detection of p24-antigen and HIV-antibodies. However, there have been few studies with limited sample collections performed regarding its sensitivity in this context [10–13], and most of them have been conducted on US-based samples, which may limit their applicability to HIV-1 subtype-B samples [14].

Recently, the development of Pre-exposure Prophylaxis (PrEP) has raised a new interest in the relevance of HRTs during HIV-1 primary infection, as PrEP associated HIV diagnosis may rely mostly on serology, even for non-TDF/FTC PrEP [15,16].

The objective of this study was to compare sensitivity and diagnostic windows of the only FDA-approved 4th generation HRT Determine with a 3rd generation HRT, ONE STEP anti-HIV (1&2) Test (InTec Products) (One-Step).

# Methods

**Sample collection**. Primary HIV-1 infection samples were collected as part of the routine activities of the Virology laboratory of the Pitié Salpêtrière Hospital (Paris, France). HIV screening was performed using two enzyme immunoassays: Alinity I HIV Ag/Ab Combo assay (Abbott, Rungis, France) and, in

case of positivity, Liaison XL Murex HIV Ag/Ab (DiaSorin, Antony, France). The Western blot New Lav Blot I (Bio-Rad laboratories, Marnes-la-Coquette, France) was used as a confirmatory assay. HIV-1 viral load was assessed using Cobas HIV-1 (Roche Diagnostics, Manheim Germany) and quantitation of p24-antigen using the enzyme immunoassay Vidas HIV p24 II (BioMerieux, Marcy l'Etoile, France) (Vidas). Viral loads, subtypes and recombinant forms of HIV-1 were assessed as previously described [17,18]. HIV primary infection was classified into three subgroups based on a simplified Fiebig staging [5]. First, the pre-seroconversion subgroup, defined as positive p24-antigen on Vidas or Liaison XL with negative Western blot and negative antibody S/CO signal on Liaison XL, or positive HIV-1 RNA and negative EIA (Stage Fiebig I). Second, an early-seroconversion group, defined by a positive p24-antigen (Liaison XL or Vidas) with a Western blot pattern compatible with HIV-1 primary infection and Liaison XL positive for HIV antibodies [19]. Lasty, the seroconversion subgroup, defined by a negative p24-antigen with a HIV-1 primary infection compatible Western blot and Liaison XL positive for HIV antibodies. HIV-1 primary infection-compatible Western blots were defined according to Fiebig's criteria as follows: they consistently presented as incomplete Western blots, either showing only one band among p24, gp41, or gp120/160, or showing at least two bands from the same set (p24, gp41, gp120/160)[20–22].

Presumptive dates of HIV-1 infection and prior PrEP use were collected from medical records. Samples were stored at -20°C prior to use.

**Samples processing.** Samples were analyzed within the same freeze-thaw cycle using the 4$^{th}$ generation HIV-screening Determine$^{TM}$ HIV Early detect (Abbott, Rungis, France) (Determine) and the 3$^{rd}$ generation HIV-screening ONE STEP anti-HIV (1&2) (InTec Products, Xiamen, China) (One-Step). Briefly, both tests are immunochromatography assays. HIV-specific antibody detection is based on gp41 and gp36 recombinant antigens for both assays, combined with a synthetic peptide of the gp36 for One-Step [23]. Samples were processed according to the respective manufacturer's guidelines, with results read by two unblinded operators after 40 minutes for Determine and 20 minutes for

One-Step. All suspected negative or trace test lines (faint or barely visible line) were adjudicated by a third, blinded operator. Samples with an invalid result were repeated once.

**Statistical analysis.** Statistical analyses and figures were performed using R (R Foundation for Statistical Computing) or Graphpad Prism (GraphPad Software, Inc). Univariate analyses were performed using Wilcoxon rank sum test for continuous variables and McNemar test for categorical variables, with $p<0.05$ considered to be statistically significant. Confidence intervals were calculated using Wilson's confidence interval for proportions. We determined a priori that considering a 90% sensitivity for each test, 180 samples would allow a clinically relevant 4% accuracy.

**Ethics.** This work was approved by the French Infectious Disease Research Ethics Board (IRB00011642), no. 2024-0107.

## Results

A total of 183 samples were included in this study. HIV-1 genetic diversity was relatively high, with approximatively 33% of HIV-1 subtype B, 18% CRF02_AG and 33% of other subtypes and circulating recombinant forms (Figure 1). One pre-seroconversion sample was repeatedly invalid by One-Step and was excluded from further analysis for this assay. The overall sensitivity was 95.1% 95% CI [90.9, 97.4] for Determine and 80.2% 95% CI [73.8, 85.4] for One-Step. As a consequence, Determine had a significantly higher sensitivity ($p = 1.38e-06$, using McNemar Test) compared to One-Step for HIV primary infection screening. As expected, there was an upward trend in sensitivity from pre-seroconversion samples, with a 71.9% 95% CI [54.6, 84.4] and 9.7% 95% CI [3.3, 24.9] sensitivities for Determine (n=32) and One-Step (n=31), respectively, to 100% 95% CI [95.5, 100] and 92.7% 95% CI [84.9, 96.6] for the early-seroconversion subgroup (n=82), and to 100% 95% CI [94.8, 100] and 97.1% 95% CI [90.2, 99.2] for the seroconversion subgroup (n=69). The results are summarized Figure 2A. Notably, Determine performed significantly better in both pre ($p = 8.57e-05$) and early ($p = 0.041$) seroconversion subgroups. The proportion of reactive results with a unique *trace* line (either Ag or

Ab for Determine) was 12.5% (4/32) and 6.4% (2/31) for Determine and One-Step, respectively, in the pre-seroconversion subgroup. These proportions were stable at 6.1% (5/82) and 9.8% (8/82) in the early-seroconversion subgroup, but lower at 0% (0/69) and 1.4% (1/69) for the seroconversion subgroup.

Of note, the results for confirmed non-subtype-B samples were only remarkably consistent with overall results. For the pre-seroconversion subgroup, Determine had a sensitivity of 66.7% 95% CI [47.4, 82.8] and One-Step a 9.5% 95% CI [2.7, 28.9], which increased to 100% 95% CI [90.8, 100] (Determine) and 92.1% [79.2, 97.3] (One-Step) for the early-seroconversion subgroup, and to 100% 95% CI [90.1, 100] (Determine) and 94.3% [81.4, 98.4] (One-Step) for the seroconversion subgroup.

Estimated dates of infection were available for 45 (24%) patients (figure 2B). During the first two weeks after infection, no assay was reactive (n=2). Between 2- and 3-weeks after infection, Determine accurately identified 78% 95% CI [45-94%] (n=7/9) of the positive samples, while One-Step identified 56% 95%CI [27-81%] (n=5/9) of them. From three weeks to one month, Determine accurately identified 90% 95%CI [62-98%] of them (n=10/11), whereas One-Step identified only 45% 95%CI [21-72%] (5/11). Beyond a month, Determine accurately identified all of them (100% sensitivity, 95%CI [86-100%], n=23/23), while One-Step was non-reactive for two samples with alleged infection dates of about one month and 70-90 days, respectively (91% sensitivity 95% CI [73-98%], n=21/23). Overall, the last negative sample occurred at 3 weeks for Determine vs 70-90 days for One-Step (Figure 2B).

Of note, six of the 183 serum samples included matched four cases of HIV infection among TDF/FTC PrEP users, in all cases due to low treatment adherence. Reported time of infection was available for two of them (three samples) and ranged from 20 to 30 days. Both rapid tests were antibody-reactive on all six serum samples.

Several reports have highlighted an elevated p24 limit of detection threshold for the Determine assay compared to Enzyme immunoassays (EIAs) [24,25]. In the present study, p24-antigen was assessed in

146 a quantitative arbitrary S/CO unit with Liaison XL and in pg/mL with Vidas. After excluding Vidas
147 samples above the limit of quantitation, both assays correlated ($r^2 = 0.81$, $p < 0.001$, Figure 3A) but
148 moderately correlated with viral loads, with a $r^2 = 0.45$ ($p < 0.0001$) and 0.63 ($p < 0.0001$) for Vidas
149 and Liaison, respectively (Figure 3 B-C). Determine p24-antigen was only reactive with highest viral
150 loads, almost consistently above 6 log copies/mL, or high p24 levels (Figure 3D, Supplementary figure
151 1). Determine p24-antigen median of positivity was 210.5 pg/mL (IQR [65.35; >400 pg/mL]). In
152 contrast to this disappointing limit of detection, both Determine and One-Step had a low limit of
153 detection for HIV antibodies, close to the Liaison XL (Supplementary figure 2). Of note, there was no
154 S/CO statistical difference between Determine and One-Step for negative, trace, low reactive and
155 reactive subgroups.

156 ## Discussion

157 Overall, this study highlights the relevance of a 4$^{th}$ HRT generation assay for primary HIV infection
158 screening, with both a higher sensitivity and a shorter diagnostic window compared with a 3$^{rd}$
159 generation HRT assay, despite an elevated detection threshold for p24-antigen compared to 4$^{th}$
160 generation EIA.

161 Study comparisons of HIV sensitivity in the context of primary infection should always be treated
162 with caution. As emphasized in this study, sensitivity is strongly influenced by the proportion of the
163 pre-seroconversion subgroup and by how pre-seroconversion is defined. This definition may be
164 based solely on a negative Western blot [7,26], which has a relatively high antibody detection
165 threshold, or on a combination of a negative Western blot with a negative-antibody 4th generation
166 EIA, an assay with a low detection threshold. To our knowledge, no study has addressed the
167 sensitivity of the One-Step assay in this subgroup using this stringent criterion. However, its
168 sensitivity is in line with previous reports from other 3$^{rd}$ generation rapid tests, such as the VIKIA
169 HIV1/2® rapid test (BioMérieux), which was non-reactive on 0% (n=2) and 15% (n=13) samples, or the
170 Autotest VIH® (AAZ-Mylan), which had a sensitivity of 0% (n=13) [27,28]. Using the other reported

definition of a negative Western blot only, 3rd generation HRTs tended to have significantly higher sensitivities, ranging from 7% to 57% [9]. However, these promising results can be misleading, as of Western-blot negative / antibody-reactive EIA samples could account for a non-negligible proportion, up to 80% of the samples tested [9,29]. With a sensitivity of 71.9% for the Determine assay in the pre-seroconversion subgroup with a 4th generation EIA and/or positive NAAT as the reference assay, our results are within the ranges of previous studies: 65% in 23 samples [30], 54% in 13 samples [28], 45.5% in 11 samples [11] as summarized in Figure 4. Of note, our higher sensitivity could be explained by a higher proportion of RNA+/EIA- samples in other studies.

Few studies have related reactive rapid tests to the diagnostic window. Pavie et al reported a window period of at least two months for five 3rd generation rapid tests in two patients [31]. Delaney et al, using commercial seroconversion panels, estimated that the median time from HIV exposure to reactivity was around 18 days for HIV 1/2 Ag/Ab Architect combo (Abbott), a reference EIA assay, 19.2 days for Determine and 26 to 32 days for 3rd generation HRTs [7]. Although our results agree with their estimates, they warn of late false non-reactive samples, which occurred in our study about a week later for Determine and about a month for One-Step.

Conflicting results have been reported for the Determine p24-antigen sensitivity in clinical samples, with sensitivities ranging from 23 to 91% compared with laboratory-based EIAs [10,29,30]. In the present study we chose a 40-minutes reading to both maximize p24-detection and get as close as possible to real-life settings, where clinicians would use the maximum incubation time to increase the likelihood of detecting a primary HIV infection. Despite our attempt to optimize this parameter, the p24 component was only 55% (63/114) sensitive compared to Liaison XL or Vidas, and was almost exclusively reactive at the highest viral loads or p24 titers. This disappointing result may be due to the high HIV subtype diversity in our samples, with widely varying p24 detection limit for non-B subtypes, as previously described EIA limitation [32]. Thus, unlike antibody detection, which tends to the limit of detection of EIA assays, further developments are needed to increase the analytical

sensitivity of HRT p24-antigen. Pending these developments, an alternative could be the use of a point-of-care NAAT test, which, despite a much higher cost, could be especially useful in the first two weeks following a potential HIV-1 infection [33].This study has several limitations. Sensitivity was performed on thawed sera, which might lead to an overestimation compared to whole blood [31]. As a consequence, point-of-care sensitivity might be lower than laboratory-based testing. Moreover, we used One-Step as a surrogate for all 3rd generation HIV rapid tests. We have previously reported sensitivities for most CE-marked 3rd generation HRT [9] on a smaller panel of other seroconversion samples. As Determine and One-Step were the two main HRT missing in this previous study, there were selected to be included in the present study. Importantly, the One-Step sensitivity matched well with other 3rd generation HRTs, which may suggest that their lack of sensitivity in this setting is directly related to a lack of HIV antibodies.

## Conclusion

Despite an elevated threshold for p24antigen detection, Determine, the first 4th generation HIV rapid test has a higher sensitivity and a shorter diagnostic window compared to 3rd generation HIV rapid tests.

## Data availability statement

The data that support the findings are available as a supplementary table.

## Author Contribution Statement

VG, AGD: Conceptualization, methodology, validation, data curation, formal analysis, resources, writing -original draft and reviewing, AN: methodology, data curation. HK, GB, PH, LP, AP, ALL: Data curation. LY: data curation, writing – review and editing.

## References

1. Alexander TS. Human Immunodeficiency Virus Diagnostic Testing: 30 Years of Evolution. Pasetti MF, editor. Clin Vaccine Immunol. **2016**; 23(4):249–253.

2. Zaaijer HL, Exel-Oehlers P v., Kraaijeveld T, Altena E, Lelie PN. Early detection of antibodies to HIV-1 by third-generation assays. The Lancet. **1992**; 340(8822):770–772.

3. Lindbäck S, Thorstensson R, Karlsson AC, et al. Diagnosis of primary HIV-1 infection and duration of follow-up after HIV exposure: AIDS. **2000**; 14(15):2333–2339.

4. Weber B, Mbargane Fall EH, Berger A, Doerr HW. Reduction of Diagnostic Window by New Fourth-Generation Human Immunodeficiency Virus Screening Assays. J Clin Microbiol. **1998**; 36(8):2235–2239.

5. Fiebig EW, Wright DJ, Rawal BD, et al. Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection. AIDS. **2003**; 17(13):1871–1879.

6. Weber B, Gürtler L, Thorstensson R, et al. Multicenter Evaluation of a New Automated Fourth-Generation Human Immunodeficiency Virus Screening Assay with a Sensitive Antigen Detection Module and High Specificity. J Clin Microbiol. **2002**; 40(6):1938–1946.

7. Delaney KP, Hanson DL, Masciotra S, Ethridge SF, Wesolowski L, Owen SM. Time Until Emergence of HIV Test Reactivity Following Infection With HIV-1: Implications for Interpreting Test Results and Retesting After Exposure. Clin Infect Dis. **2017**; 64(1):53–59.

8. Adams S, Luo W, Wesolowski L, et al. Performance evaluation of the point-of-care INSTI™ HIV-1/2 antibody test in early and established HIV infections. Journal of Clinical Virology. **2017**; 91:90–94.

9. Guiraud V, Beaulieu Q, Fauchois A, et al. Rapid tests should be used with caution for HIV-1 primary infection screening. Med Microbiol Immunol. **2024**; 213(1):10.

10. Fitzgerald N, Cross M, O'Shea S, Fox J. Diagnosing acute HIV infection at point of care: a retrospective analysis of the sensitivity and specificity of a fourth-generation point-of-care test for detection of HIV core protein p24. Sex Transm Infect. **2017**; 93(2):100–101.

11. Mourez T, Lemée V, Delbos V, et al. HIV rapid screening tests and self-tests: Be aware of differences in performance and cautious of vendors. EBioMedicine. **2018**; 37:382–391.

12. Parker MM, Bennett SB, Sullivan TJ, et al. Performance of the Alere Determine™ HIV-1/2 Ag/Ab Combo Rapid Test with algorithm-defined acute HIV-1 infection specimens. Journal of Clinical Virology. **2018**; 104:89–91.

13. Masciotra S, Luo W, Westheimer E, et al. Performance evaluation of the FDA-approved Determine™ HIV-1/2 Ag/Ab Combo assay using plasma and whole blood specimens. Journal of Clinical Virology. **2017**; 91:95–100.

14. Ly TD, Ebel A, Faucher V, Fihman V, Laperche S. Could the new HIV combined p24 antigen and antibody assays replace p24 antigen specific assays? Journal of Virological Methods. **2007**; 143(1):86–94.

15. Raphael J. Landovitz, Emily Voldal, Brett Hanscom, Susan H. Eshleman, Estelle Piwowar-Manning, Philip Sullivan, Marybeth McCauley, Lydia Soto-Torres, James F. Rooney, Alex R. Rinehart, Myron S. Cohen, Mina Hosseinipour, Sinead Delany-Moretlwe, Beatriz Grinsztejn. Site-Based HIV Testing Assay Performance for Cabotegravir and TDF-FTC PrEP Failure in HPTN 083 [Internet]. CROI - Conference on Retroviruses and Opportunistic Infections; 2024 [cited

2024 Sep 3]. Available from: https://www.croiconference.org/abstract/site-based-hiv-testing-assay-performance-for-cabotegravir-and-tdf-ftc-prep-failure-in-hptn-083/

16. Bekker L-G, Das M, Abdool Karim Q, et al. Twice-Yearly Lenacapavir or Daily F/TAF for HIV Prevention in Cisgender Women. N Engl J Med. **2024**; :NEJMoa2407001.

17. Guiraud V, Bocobza J, Desmonet M, et al. Are Confirmatory Assays Reliable for HIV-1/HIV-2 Infection Differentiation? A Multicenter Study. Hayden R, editor. J Clin Microbiol. **2023**; :e00619-23.

18. HIV French Resistance website [Internet]. 2024 [cited 2024 Sep 3]. Available from: https://hivfrenchresistance.org/protocols/

19. AIDS: proposed WHO criteria for interpreting western blot assays for HIV-1, HIV-2, and HTLV-I/HTLV-II. Bull World Health Organ. **1991**; 69(1):127–129, 131–133.

20. Profil atypique de western blot VIH-1 (New Lav Blot I) avec perte d'anticorps anti-gp120, anti-gp41 et anti-polymérase au cours d'une infection par du VIH-1 sous-type A au stade sida: Virologie. **2022**; 26(3):253–255.

21. Saah AJ, Farzadegan H, Fox R, et al. Detection of early antibodies in human immunodeficiency virus infection by enzyme-linked immunosorbent assay, Western blot, and radioimmunoprecipitation. J Clin Microbiol. **1987**; 25(9):1605–1610.

22. Tuaillon E, Sanosyan A, Pisoni A, Liscouët J, Makinson A, Perre PVD. Staging of recent HIV-1 infection using Geenius rapid confirmatory assay compared to INNO-LIA, New Lav and Blot 2.2 assays. Journal of Clinical Virology. **2017**; 95:47–51.

23. Guiraud V, Gauthier V, Lazga H, Leroy I, Gautheret-Dejean A. HIV rapid tests immunological internal control can be misleading. Annales Pharmaceutiques Françaises. **2024**; 82(5):765–770.

24. Stone M, Bainbridge J, Sanchez AM, et al. Comparison of Detection Limits of Fourth- and Fifth-Generation Combination HIV Antigen-Antibody, p24 Antigen, and Viral Load Assays on Diverse HIV Isolates. Caliendo AM, editor. J Clin Microbiol. **2018**; 56(8):e02045-17.

25. Agence Nationale de Sécurité du médicament et des produits de santé. Test rapide Alere HIV Combo - Alere - Information - Recommandation ANSM [Internet]. 2018 [cited 2024 Jun 30]. Available from: https://ansm.sante.fr/informations-de-securite/test-rapide-alere-hiv-combo-alere-information-recommandation-ansm

26. Patel P, Bennett B, Sullivan T, Parker MM, Heffelfinger JD, Sullivan PS. Rapid HIV screening: Missed opportunities for HIV diagnosis and prevention. Journal of Clinical Virology. **2012**; 54(1):42–47.

27. Gomes C, Azevedo-Pereira JM. The performance of the VIKIA® HIV1/2 rapid test—Evaluation of the reliability and sensitivity. Journal of Virological Methods. **2011**; 173(2):353–356.

28. Delaugerre C, Antoni G, Mahjoub N, et al. Assessment of HIV Screening Tests for Pre-Exposure Prophylaxis Programs. The Journal of Infectious Diseases [Internet]. **2017** [cited 2024 Jul 2]; . Available from: https://academic.oup.com/jid/article-lookup/doi/10.1093/infdis/jix297

29. Wratil PR, Rabenau HF, Eberle J, et al. Comparative multi-assay evaluation of Determine™ HIV-1/2 Ag/Ab Combo rapid diagnostic tests in acute and chronic HIV infection. Med Microbiol Immunol. **2020**; 209(2):139–150.

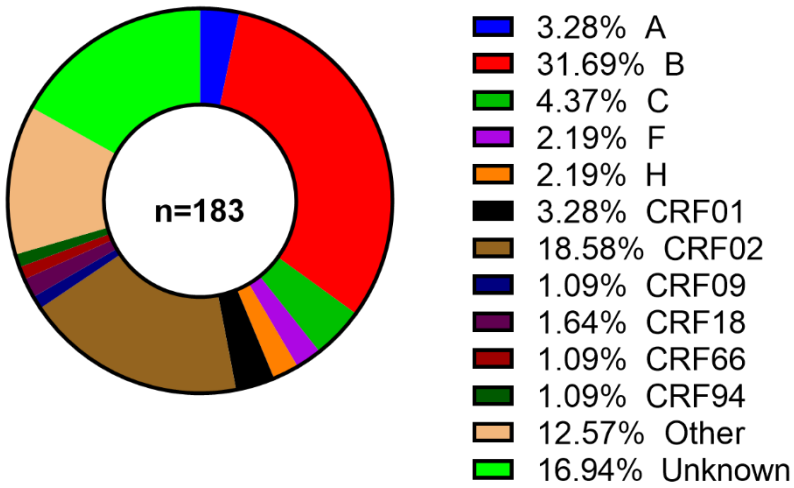
30. Van Tienen C, Rugebregt S, Scherbeijn S, Götz H, Geurts Van Kessel C. The performance of the Alere HIV combo point-of-care test on stored serum samples; useful for detection of early HIV-1 infections? Sex Transm Infect. **2018**; 94(5):331–333.

31. Pavie J, Rachline A, Loze B, et al. Sensitivity of Five Rapid HIV Tests on Oral Fluid or Finger-Stick Whole Blood: A Real-Time Comparison in a Healthcare Setting. Pai NP, editor. PLoS ONE. **2010**; 5(7):e11581.

32. Qiu X, Sokoll L, Duong Ly T, et al. An improved HIV antigen/antibody prototype assay for earlier detection of acute HIV infection. Journal of Clinical Virology. **2021**; 145:105022.

33. Jordan JA, Plantier JC, Templeton K, Wu AHB. Multi-site clinical evaluation of the Xpert® HIV-1 viral load assay. Journal of Clinical Virology. **2016**; 80:27–32.

34. Visseaux B, Assoumou L, Mahjoub N, et al. Surveillance of HIV-1 primary infections in France from 2014 to 2016: toward stable resistance, but higher diversity, clustering and virulence? Journal of Antimicrobial Chemotherapy. **2019**; :dkz404.




315

316 **Figure 1**: HIV-1 subtypes and Circulating Recombinant Forms of serum samples included in this study.

317

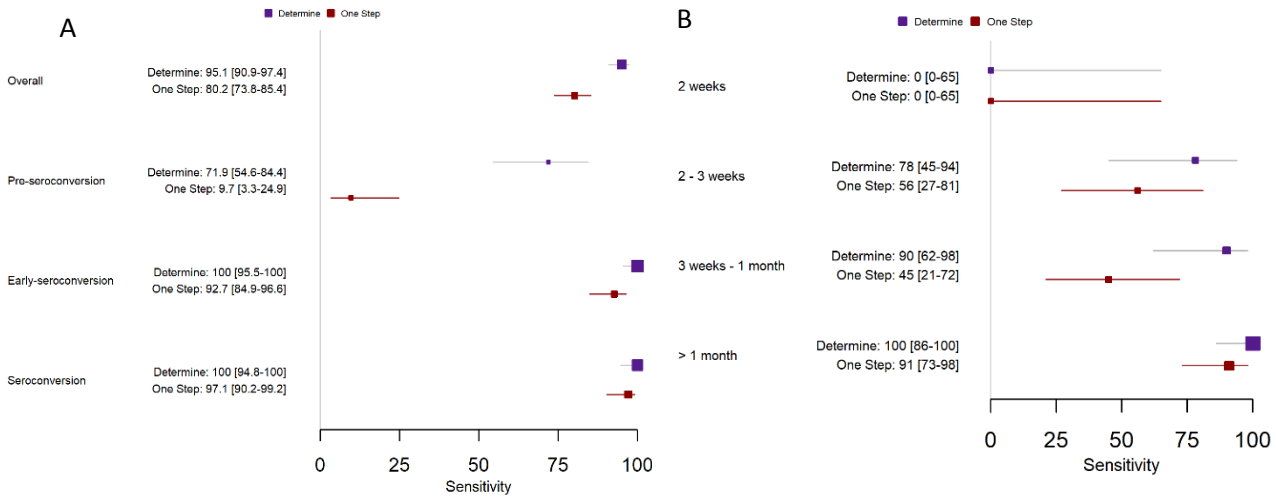



**Figure 2**: Determine and One-Step sensitivity (%) according to seroconversion subgroups (A) or time from infection (B).

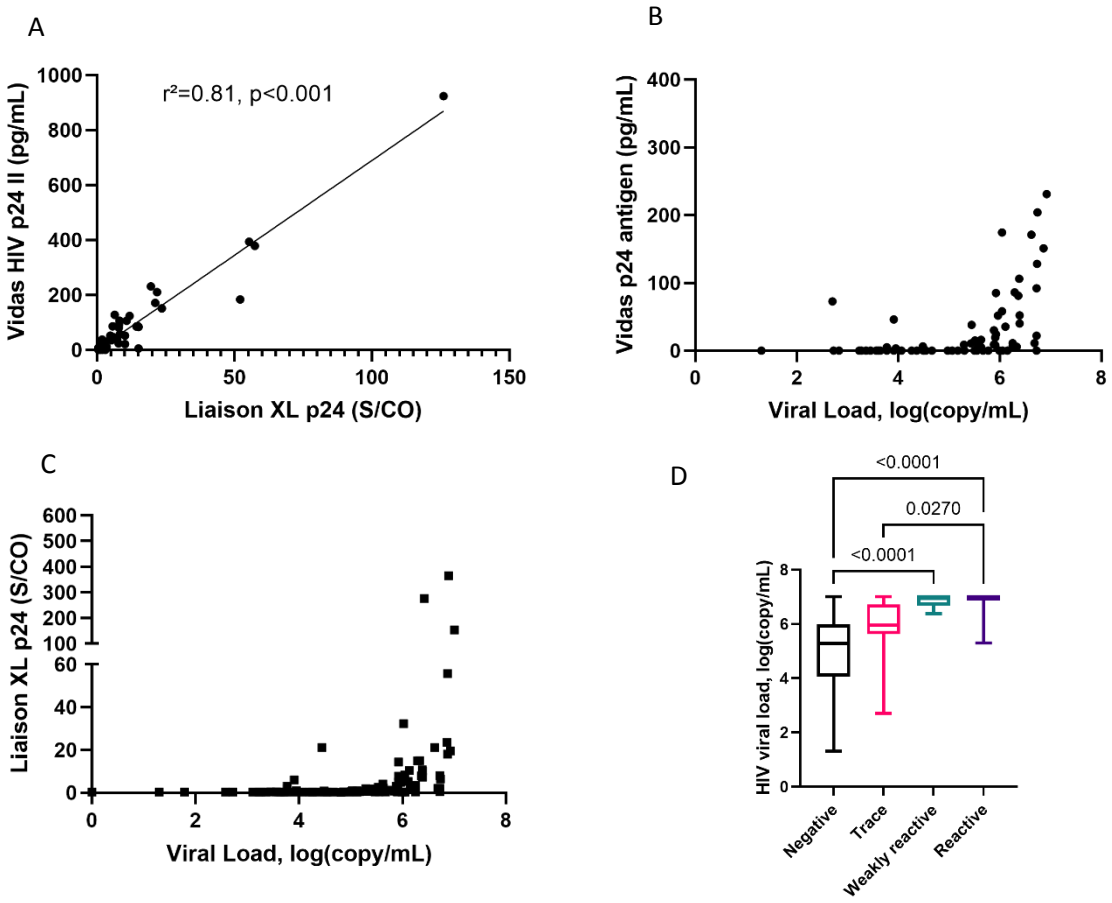
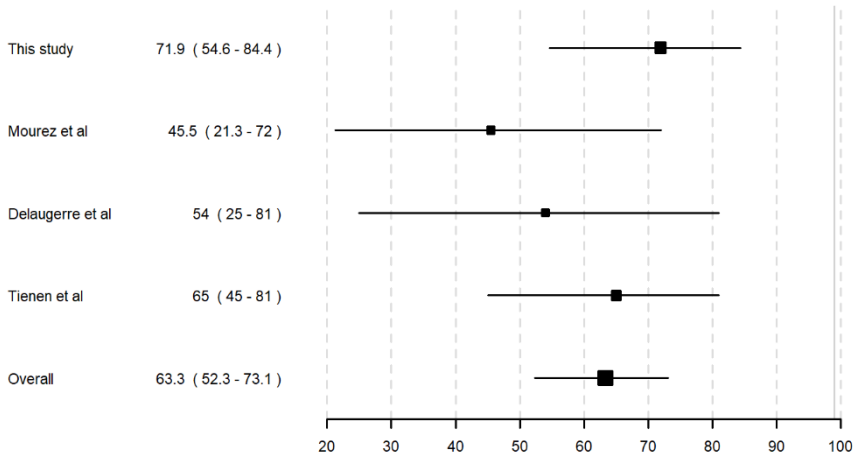



**Figure 3**: p24 antigen determination in serum is dependent on the assay and is poorly correlated with HIV viral loads. Correlation between Vidas HIV p24 II and Liaison XL p24 (A), between Vidas p24 antigen and Viral load (B), between Liaison XL and Viral load (C), and between Determine and Viral load (D). p24 values above limit of quantification were excluded from the figures. p24 was expressed in pg/mL for the Vidas assay, in a quantitative S/CO value for the Liaison XL assay, and as a visual intensity for Determine. Viral loads are expressed as log(copy/mL). Comparisons were performed using Wilcoxon rank sum (D), and Spearman coefficient was used for correlation (A).

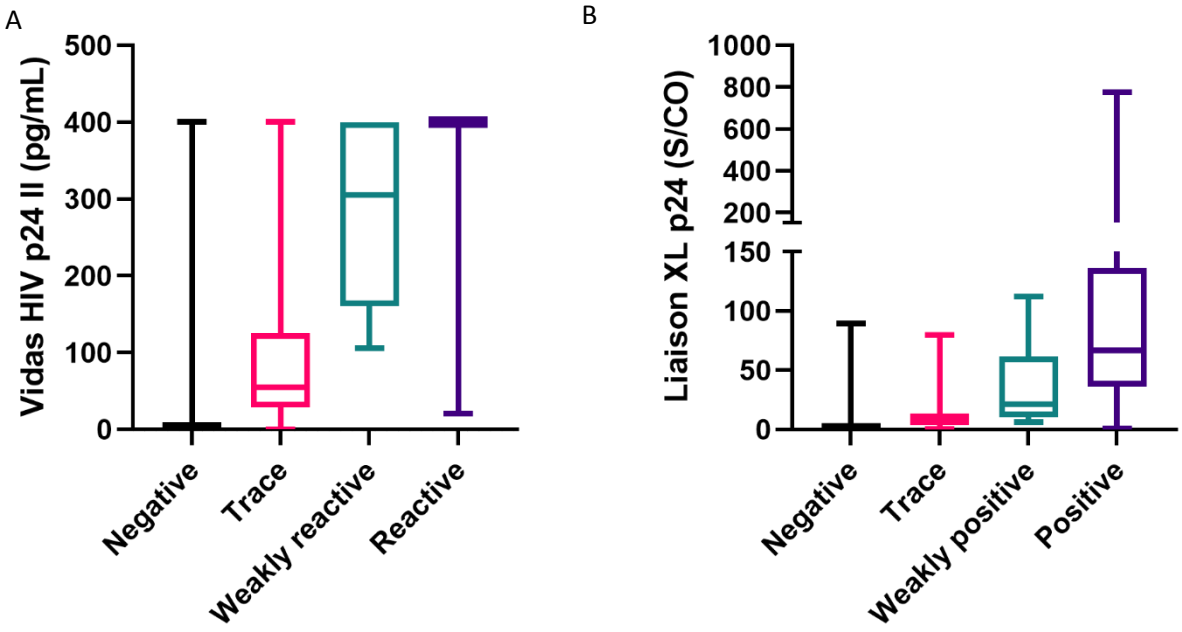



358

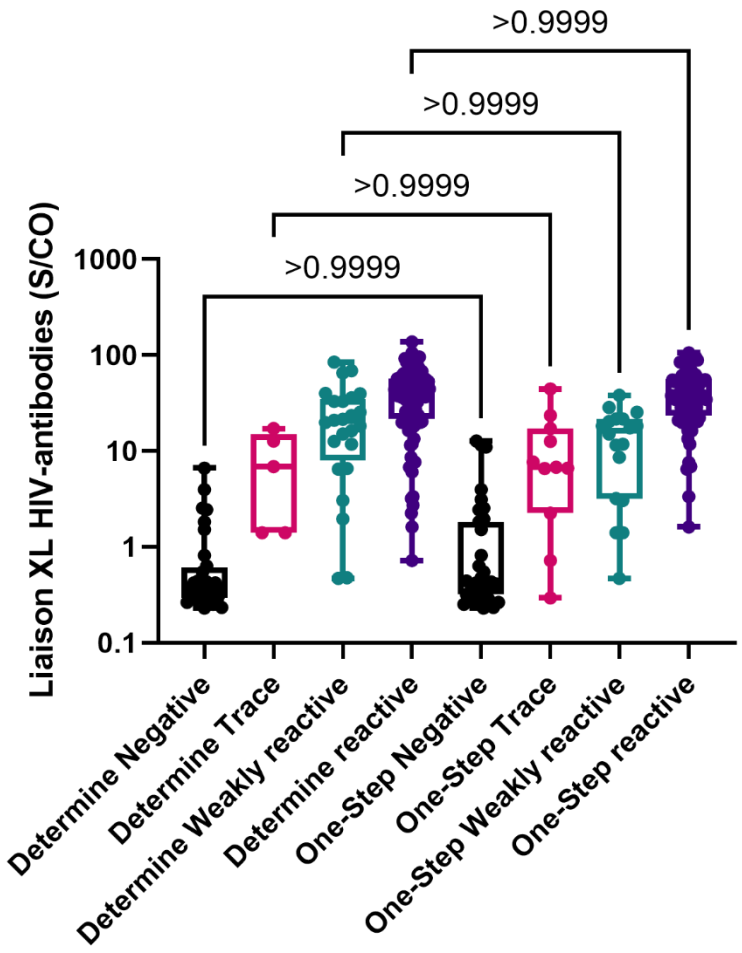
359 **Figure 4:** Comparison of pre-seroconversion subgroup sensitivity of the Determine assay with
360 previous studies.

361

362




**Supplementary Figure 1**: Determine p24 antigen band intensity according to p24 quantitation in serum using Vidas (A) or Liaison XL (B). Band intensities were defined as follow. Reactive: Test line at least as visible as the Control line, Weakly reactive: Test line less reactive than the control line but positive without doubt. Trace: barely visible Test line. Negative: no Test line.




**Supplementary Figure 2**: Determine (antibody) and One-Step Test line visual intensities according to Liaison XL S/CO HIV-antibody ratio. Numbers in the figure represent p-values, assessed using Wilcoxon-rank sum.