Immunoblots may not be effective in confirming the recency of HIV-1 infection

Alice-Andrée Mariaggi, Elise Gardiennet, Karl Stefic, Asma Essat, Antoine Cheret, Cécile Goujard, Laurence Meyer, Francis Barin, Véronique Avettand-Fenoel

HAL Id: hal-03256051

https://u-paris.hal.science/hal-03256051

Submitted on 13 Feb 2023

1   **Immunoblots may not be effective in confirming the recency of HIV-1 infection**

2

3   Alice-Andrée MARIAGGI [a,b,c], Elise GARDIENNET [a,b], Karl STEFIC [d], Asma ESSAT [e],

4   Antoine CHERET [a,b,f], Cécile GOUJARD [e,f], Laurence MEYER [e], Francis BARIN [d],

5   Véronique AVETTAND-FENOEL[a,b,g,§], for the ANRS PRIMO cohort

6

7   [a] Université Paris Descartes, Sorbonne Paris Cité, Faculté de Médecine, Paris, France

8   [b] INSERM, U1016, CNRS, UMR8104, Institut Cochin, Paris, France

9   [c] APHP, Laboratoire de Virologie, CHU Cochin, Paris, France

10   [d] CHU Bretonneau & Université François Rabelais, Centre National de Référence du VIH-
11   Laboratoire Associé, Inserm U1259, Tours, France

12   [e] INSERM CESP U1018, University Paris Saclay, Hôpital Bicêtre, Assistance Publique-
13   Hôpitaux de Paris (APHP), Le Kremlin-Bicêtre, France

14   [f] Service de Médecine Interne et d'Immunologie Clinique, Hôpital Bicêtre, Assistance
15   Publique-Hôpitaux de Paris (APHP), Le Kremlin-Bicêtre

16   [g] APHP, Laboratoire de Microbiologie Clinique, Hôpital Necker-Enfants Malades, Paris,
17   France

18   [§] Corresponding author: Véronique Avettand-Fenoel

19   Laboratoire de Microbiologie Clinique, 149 rue de Sèvres, 75015 Paris, France

20   veronique.avettand@aphp.fr (VAF)

21

22

23 **<u>Abstract</u>**

24

25 Recently, immunoblots (IBs) have tended to substitute Western blots (WBs) for HIV infection

26 diagnosis. Several studies have confirmed IBs' high sensitivity to confirm HIV infection for

27 every stage. Since the nature and pattern of the antigens of IBs are different from those of

28 WB, the abilities of IBs and WBs to distinguish the stages of recent seroconversion and open-

29 ended chronic infection might differ. We aimed to evaluate the performance of two IBs

30 (INNO-LIA™ HIVI/II, Fujirebio, and Geenius™ HIV1/2 Confirmatory assay, Bio-Rad) to

31 define the stage of infection. We studied 53 patients from the French ANRS CO6 PRIMO

32 cohort. IBs have higher positive rates than WB. However, Geenius was less sensitive than

33 WB and INNO-LIA to detect antibodies to p31 (0% vs 22.6% and 15.1%, respectively), so it

34 could wrongly label late Fiebig stage and open-ended chronic infections as recent infections

35 (n=5/53). For the first time, we provide evidence that centralized WBs associated with an

36 enzyme immunoassay for the identification of recent HIV-1 infection support the

37 establishment of a more accurate diagnosis of primary HIV infection to improve the accuracy

38 of enrollments in cohorts of recent HIV infections useful for epidemiological studies,

39 pathogenesis studies or therapeutic trials.

40

49

50 **<u>Highlights :</u>**

51 • Evaluation of Immunoblots to precise the recency of HIV infection.

52 • 53 patients from the ANRS PRIMO cohort.

53 • Geenius is not efficient at detecting anti-p31 antibodies in Fiebig VI.

54 • Immunoblots could wrongly label late Fiebig stages as more recent infections.

55 • Centralized WBs improve the accuracy of enrollments in primary infections cohorts.

56

57

58

Introduction:

Diagnosis of human immunodeficiency virus (HIV) infection as soon as the primary HIV infection (PHI) occurs is essential for early initiation of combined antiretroviral therapy (cART) to preserve immune function, limit the viral reservoir, and prevent transmission. The biological characterization of PHI is also useful for epidemiological studies as well as enrollment in cohorts dedicated to pathogenesis studies or therapeutic trials (Colby et al., 2018; Dong et al., 2018).

For the laboratory diagnosis of HIV infection, reactive serological testing with a 4th generation enzyme immunoassay (EIA) must be confirmed. The confirmatory assays can be Western blots (WBs) using viral antigens, which are considered the gold standard to confirm HIV infection or immunoblots (IBs) using recombinant or synthetic antigens. In case of discrepancy between EIA and confirmatory assays, HIV-RNA plasma detection is recommended.

PHI infection usually refers to acute HIV infection and the early stage of HIV infection up to about six months but definitions and biological markers may differ across study. French guidelines defined PHI as detectable HIV-RNA associated with a negative 4th generation EIA or a positive EIA with ≤ 5 HIV-specific antibodies on WB (Morlat, 2019). WB results are then useful to precisely determine the stage of PHI based on Fiebig's classification (Fiebig et al., 2003). Six stages are defined according to the dynamics of HIV-RNA, p24 antigen and antibody seroconversions observed from WB results. This broadly used classification distinguishes acute infection (stages I–III), recent seroconversion (stages IV–V), and open-ended chronic infection (stage VI). Stages I–III are defined by a negative WB. Stage IV is defined as the presence of HIV-1-specific bands that fail to meet criteria for a reactive WB, identified by the US Food and Drug Administration as reactivity to 2 of the following

83 antigens: p24, gp41, and gp120/160. Fiebig stage V is defined as a reactive pattern (presence

84 of at least 2 antigens) but lacking p31 reactivity. Stage VI is defined as full reactivity,

85 including a p31 band.

86 In recent years, several new IBs, which are faster to use than WBs, have tended to substitute

87 WBs as confirmation assays. IBs assess fewer antibodies than WBs, but their high sensitivity

88 to confirm HIV infection has been reported in several studies (Kondo et al., 2018; Lindman et

89 al., 2019; Serhir et al., 2019; Tinguely et al., 2014). However, their ability to differentiate PHI

90 from later stages needs to be largely evaluated.

91 We aimed to evaluate two IBs frequently used at preinclusion in the French ANRS (Agence

92 Nationale de Recherches sur le SIDA et les Hépatites Virales) CO6 PRIMO cohort, to

93 determine if they are performant to consider a patient as recently HIV infected.

94

95 **Materials and methods**

96 Fifty-three patients from the ANRS PRIMO cohort between November 2018 and May 2019

97 who had frozen blood plasma at inclusion in the cohort participated in this study. The cohort

98 was approved by the Ile-de-France-3 Ethics Committee, and patients gave their informed

99 consent to participate. Participants presenting with acute or recent HIV-1 infection,

100 symptomatic or not, were included if they presented one of the following criteria: 1)

101 detectable p24 antigenemia or plasma viral load, associated with an incomplete confirmatory

102 assay (i.e., indeterminate or positive confirmatory assay with absence of antibodies to p31

103 and/or p68) during the six weeks before inclusion, 2) detectable p24 antigenemia or plasma

104 viral load, with either a negative or weakly reactive EIA or a negative WB or IB during the

105 six weeks before inclusion, or 3) an interval of <3 months between a negative and a positive

106 EIA (Goujard et al., 2006). Preinclusion was based on clinical investigation and routine

107 laboratory assays, which could vary depending on the laboratory. Subsequently, centralized

108 Vidas® HIV-DUO Ultra and WBs were systematically performed on inclusion samples

109 (APHP, Hôpital Necker-Enfants Malades, Paris, France). Vidas® HIV-DUO Ultra, which is

110 an Enzyme Linked Fluorescent Assay (ELIFA), was applied to determine if antibodies against

111 HIV-1 or HIV-2 or p24 antigen were detectable.

112 Bio-Rad HIV-1 WB uses the inactivated HIV-1 native viral antigens Env (gp160, gp120 and

113 gp41), Pol (p68/65, p51 and p31) and Gag (p55, p40/39, p24, and p18/17). The assay was

114 performed according to the manufacturer's recommendations using a visual reading. We

115 interpreted the WBs according to the WHO's recommendations (WHO, 1991): they were

116 considered positive if at least two antibodies against the envelope antigens were detected. The

117 WBs were indeterminate if antibodies were detected without the above criteria of positivity.

118 As a result, we were able to precisely determine the pattern of HIV-specific antibodies on WB

119 to classify the stage of infection with centralized tools.

120 The two IB assays INNO-LIA™ HIVI/II (Fujirebio) and Geenius™ HIV1/2 Confirmatory

121 assay (Bio-Rad) were performed by the same operator on the same inclusion samples and

122 interpreted according to manufacturer recommendations. The INNO-LIA assay is a single-use

123 line immunoassay (LIA) which can detect antibodies against five HIV-1 proteins (gp120,

124 gp41, p31, p24, and p17) and two against HIV-2 envelope proteins (gp105 and gp36). Patients

125 samples were incubated all night with the test strips. Each line's intensity was read visually

126 and compared to control lines. INNO-LIA was interpreted as HIV-1 positive when there were

127 at least two positive anti-HIV-1 antibodies, including one directed against the HIV-1 envelope

128 antigens. The Geenius assay is a single-use immunochromatographic test. It is composed of

129 four HIV-1 antigens (Env gp120 and gp41, Pol p31 and Gag p24 and p17) and two HIV-2

130 antigens (gp36 and gp140). The duration of the assay is less than one hour. In our study,

131   bands were observed visually. Geenius was interpreted HIV-1 positive if the antibody to at

132   least one envelope protein and antibody to another HIV antigen were positive.

133   Fiebig's classification (Fiebig et al., 2003) was used to differentiate acute and early infection

134   according to Vidas® HIV-DUO Ultra, WB and IB assays.

135   Finally, a centralized EIA was also performed for the identification of recent HIV-1 infection

136   (EIA-RI) to clarify doubtful cases. It was based on the quantification of antibodies binding to

137   synthetic antigens of the immunodominant epitope of gp41 and the V3 region of gp120 (Barin

138   et al., 2005). A score <0.5 was in favor of infection of fewer than 180 days, as previously

139   described (Barin et al., 2005).

140   Fisher and χ2 tests were used to compare WB and IBs and to evaluate the relationship

141   between WB or IB results and Fiebig stages (Prism version 8.0, GraphPad).

142

143   **Results**

144   Fifty-three patients were tested with WB and two IBs. The positive rate of WB (13.2%) was

145   lower than that of INNO-LIA (66.0%, p<0.0001) and Geenius (62.3%, p<0.0001). INNO-LIA

146   and Geenius showed positive results for most of the indeterminate WB samples, 87.1%

147   (n=27/31) and 77.4% (n=24/31), respectively (Table 1). Seven samples showed concordant

148   positive results with the three assays (n=7/53, 13.2%).

149   Antibody patterns help to distinguish acute, recent, and established infections. Antibodies to

150   p24 and envelope glycoproteins are the earliest to develop during seroconversion. WB and

151   INNO-LIA better detected antibodies to p24 than Geenius (66.0% vs 9.4%, p<0.0001 and

152   62.3% vs 9.4%, p<0.0001, respectively). Detection of antibodies to Env antigens was higher

153   with Geenius than with WB (gp120/160: 62.3% vs 30.2%, p =0.0017; gp41: 84.9% vs 13.2%,

154   p<0.001). Antibodies to gp120/160 were more often detected with Geenius than with INNO-

155   LIA (62.3% vs 32.1%, p= 0.0033). Antibodies to p31 appear later during the course of

156   infection and are used to define Fiebig stage VI. WB detected antibodies to p31 in twelve

157   samples (n=12/53, 22.6%), including eight samples that were also p31 positive with INNO-

158   LIA (n=8/53, 15.1%). Both WB and INNO-LIA better detected antibodies to p31 than

159   Geenius, which did not detect any antibodies to p31 in any sample (WB vs. Geenius: p =

160   0.0002; INNO-LIA vs Geenius: p=0.0059) (Table 1).

161   Fiebig classification applied to Vidas® HIV-DUO Ultra combined with either WB, INNO-

162   LIA, or Geenius showed different distributions of Fiebig stages (p<0.0001) (Table 1).

163   Differences were particularly observed for the latest Fiebig stages IV to VI. Fiebig

164   classification according to WB ranked more samples in Fiebig stage IV (n=31/53, 58.5%)

165   than both INNO-LIA (n=9/53, 17.0%) and Geenius (n=12/53, 22.6). Conversely, the IBs

166   ranked more samples in Fiebig stage V than WB (INNO-LIA n=27/53, 50.9%, Geenius

167   n=33/53, 62.3% vs WB n= 1/53, 1.9%). Due to the difference in detecting antibodies to p31,

168   WB and INNO-LIA classified some samples in Fiebig stage VI (n=6/53, 11.3%, and n=8/53,

169   15.1%, respectively), whereas Geenius did not.

170   We further performed EIA-RI to assess discrepancies. Out of 53 samples, 48 were classified

171   as recent infection shorter than 180 days (median score: 0.01, range [0.01-0.25]), one

172   equivocal and four long-term (>180 days). These five cases are detailed on Table 2. The only

173   sample presenting Fiebig stage V characteristics on WB showed an index of EIA-RI at 0.99 in

174   favor of an infection longer than 180 days. The IBs also classified this sample in Fiebig stage

175   V. INNO-LIA had been used to confirm the diagnosis at preinclusion for this participant and

176   presented negative for antibodies to p31 and positive for antibodies to p24. There was no

177   previous negative HIV serology and no p24 detection, and the CD4/CD8 T cell ratio was 0.2,

178   with 4.7 log copies HIV-RNA/mL at inclusion. Immunosuppression in late chronic infection

179   leading to loss of antibodies to Pol and Gag might have distorted the confirmatory assay and

180   mislead the Fiebig classification.

181   Three patients out of six classified in Fiebig stage VI with WB had an elevated index of EIA-

182   RI >0.8 in favor of infection longer than 180 days. They all had been diagnosed on the basis

183   of the results of a Geenius assay at preinclusion (no antibodies to p24 or p31), and two of

184   them were positive for p24 antigen at preinclusion. Curiously, one patient had very low HIV-

185   RNA and HIV-DNA and a CD4/CD8 T cell ratio conserved >1 at inclusion fourteen days

186   later. This patient could be a natural HIV controller. The fifth patient presented a doubtful

187   EIA-RI index (0.44) and was positive for all three assays but with antibodies to p31 detected

188   only with WB. The patient had no past serology and no p24 antigen detected, and WB was

189   complete except for gp110.

190

191   **Discussion**

192   Different manufacturers have approved several IB assays as confirmation assays, without any

193   doubt regarding their performance (Kondo et al., 2018; Serhir et al., 2019; Tinguely et al.,

194   2014), but few studies concern their ability to distinguish infection stages. Therefore, our aim

195   was to focus on acute and recent stages.

196   The present study on a large number of samples clearly shows that the ability to detect each

197   antibody differed between the IBs and WB. The higher ability of the IBs to detect anti-Env

198   antibodies led to a higher positive rate of these assays than that of WB, which is advantageous

199   to confirm HIV diagnosis as soon as the earliest Fiebig stages of seroconversion. WBs were

200   then more often indeterminate, with a profile suggesting recent infection. Moreover, Gag and

201   Pol antibodies are essential to distinguish acute from recent and chronic infections,

202   particularly anti-p31 antibodies, which characterize Fiebig stage VI. Our study showed that

203   Geenius is not efficient at detecting antibodies to p31 in Fiebig stages VI, so Geenius could

204   wrongly label late Fiebig stage and chronic infections as more recent infections. These data

205   are in agreement with the lower capacity of Geenius to detect anti-p24 and anti-p31 antibodies

206   observed during chronic HIV infection (Tuaillon et al., 2017). An alternative method based on

207   the quantitative measurements of antibody band intensities determined by the automated

208   Geenius optical reader may have an interest to distinguish between recent and long-standing

209   HIV infection, as previously suggested (Keating et al., 2016).

210   Overall, we provide evidence that Geenius and INNO-LIA may not be assays of choice to

211   stage infections following Fiebig classification. As the latest stages or some rare cases, such

212   natural HIV controllers, can be confusing for both WB and the IBs, the EIA-RI result,

213   interpreted in association with other assays, can be an additional argument to confirm or reject

214   recent infection.

215   In conclusion, our study highlights the difficulties of providing consistent results for

216   determining the stage of HIV infection when antibodies are already detectable. Clinical

217   laboratories must keep in mind that Geenius might mistakenly refer to recent infection, so if a

218   recent infection is suspected, additional investigations are needed. In our study, we showed

219   that a centralized WB confirmation with a complementary assay of recent infection combined

220   with assays realized at preinclusion are beneficial to confirm the recency of HIV infection and

221   reassure enrollment criteria in PHI cohorts.

222

228

229   **Declarations of interest**: none
230

231

232   **References**

233   Barin, F., Meyer, L., Lancar, R., Deveau, C., Gharib, M., Laporte, A., Desenclos, J.C. and
234   Costagliola, D., 2005. Development and validation of an immunoassay for
235   identification of recent human immunodeficiency virus type 1 infections and its use on
236   dried serum spots. J Clin Microbiol 43, 4441-7.
237   Colby, D.J., Trautmann, L., Pinyakorn, S., Leyre, L., Pagliuzza, A., Kroon, E., Rolland, M.,
238   Takata, H., Buranapraditkun, S., Intasan, J., Chomchey, N., Muir, R., Haddad, E.K.,
239   Tovanabutra, S., Ubolyam, S., Bolton, D.L., Fullmer, B.A., Gorelick, R.J., Fox, L.,
240   Crowell, T.A., Trichavaroj, R., O'Connell, R., Chomont, N., Kim, J.H., Michael, N.L.,
241   Robb, M.L., Phanuphak, N., Ananworanich, J. and group, R.V.s., 2018. Rapid HIV
242   RNA rebound after antiretroviral treatment interruption in persons durably suppressed
243   in Fiebig I acute HIV infection. Nat Med 24, 923-926.
244   Dong, K.L., Moodley, A., Kwon, D.S., Ghebremichael, M.S., Dong, M., Ismail, N., Ndhlovu,
245   Z.M., Mabuka, J.M., Muema, D.M., Pretorius, K., Lin, N., Walker, B.D. and Ndung'u,
246   T., 2018. Detection and treatment of Fiebig stage I HIV-1 infection in young at-risk
247   women in South Africa: a prospective cohort study. Lancet HIV 5, e35-e44.
248   Fiebig, E.W., Wright, D.J., Rawal, B.D., Garrett, P.E., Schumacher, R.T., Peddada, L.,
249   Heldebrant, C., Smith, R., Conrad, A., Kleinman, S.H. and Busch, M.P., 2003.
250   Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications
251   for diagnosis and staging of primary HIV infection. AIDS 17, 1871-9.
252   Goujard, C., Bonarek, M., Meyer, L., Bonnet, F., Chaix, M.L., Deveau, C., Sinet, M.,
253   Galimand, J., Delfraissy, J.F., Venet, A., Rouzioux, C., Morlat, P. and Agence
254   Nationale de Recherche sur le Sida, P.S.G., 2006. CD4 cell count and HIV DNA level
255   are independent predictors of disease progression after primary HIV type 1 infection
256   in untreated patients. Clin Infect Dis 42, 709-15.
257   Keating, S.M., Kassanjee, R., Lebedeva, M., Facente, S.N., MacArthur, J.C., Grebe, E.,
258   Murphy, G., Welte, A., Martin, J.N., Little, S., Price, M.A., Kallas, E.G., Busch, M.P.
259   and Pilcher, C.D., 2016. Performance of the Bio-Rad Geenius HIV1/2 Supplemental
260   Assay in Detecting "Recent" HIV Infection and Calculating Population Incidence. J
261   Acquir Immune Defic Syndr 73, 581-588.
262   Kondo, M., Sudo, K., Sano, T., Kawahata, T., Itoda, I., Iwamuro, S., Yoshimura, Y.,
263   Tachikawa, N., Kojima, Y., Mori, H., Fujiwara, H., Hasegawa, N. and Kato, S., 2018.
264   Comparative evaluation of the Geenius HIV 1/2 Confirmatory Assay and the HIV-1
265   and HIV-2 Western blots in the Japanese population. PLoS One 13, e0198924.
266   Lindman, J., Honge, B.L., Kjerulff, B., Medina, C., da Silva, Z.J., Erikstrup, C., Norrgren, H.
267   and Mansson, F., 2019. Performance of Bio-Rad HIV-1/2 Confirmatory Assay in
268   HIV-1, HIV-2 and HIV-1/2 dually reactive patients - comparison with INNO-LIA and
269   immunocomb discriminatory assays. J Virol Methods 268, 42-47.
270   Morlat, P. 2019. Prise en charge médicale des personnes vivant avec le VIH
271   Recommandations du groupe d'expert, Conseil National du Sida et des Hépatites
272   Virales - Agence Nationale de Recherche sur le SIDA_VIH et les Hépatites
273   Serhir, B., Desjardins, C., Doualla-Bell, F., Simard, M., Tremblay, C. and Longtin, J., 2019.
274   Evaluation of the Bio-Rad Geenius HIV 1/2 Assay as Part of a Confirmatory HIV

275   Testing Strategy for Quebec, Canada: Comparison with Western Blot and Inno-Lia
276   Assays. J Clin Microbiol 57.
277 Tinguely, C., Schild-Spycher, T., Bahador, Z., Gowland, P., Stolz, M. and Niederhauser, C.,
278   2014. Comparison of a conventional HIV 1/2 line immunoassay with a rapid
279   confirmatory HIV 1/2 assay. J Virol Methods 206, 1-4.
280 Tuaillon, E., Sanosyan, A., Pisoni, A., Liscouet, J., Makinson, A. and Perre, P.V., 2017.
281   Staging of recent HIV-1 infection using Geenius rapid confirmatory assay compared
282   to INNO-LIA, New Lav and Blot 2.2 assays. J Clin Virol 95, 47-51.
283 WHO, 1991. AIDS : proposed WHO criteria for interpreting Western blot assays for HIV-1,
284   HIV-2, and HTLV-I/HTLV-Ila. Bulletin of the World Health Organization 69 (1):
285   127-130.
286

287
288
289

Table 1. Comparison of positive antibodies and stages of Fiebig's classification between Western blot (WB), Geenius, and INNO-LIA

N (%)	Assay results			Positive antibodies				Fiebig stages					
	Positive	Indeter-minate	Negative	GP160/120	GP41	P24	P31	I	II	III	IV	V	VI
Western blot, Bio-Rad	7 (13.2)	31 (58.5)	15 (28.3)	16 (30.2)	7 (13.2)	35 (66.3)	12 (22.6)	0 (0.0)	10 (18.9)	5 (9.4)	31 (58.5)	1 (1.9)	6 (11.3)
Immunoblot, INNO-LIA HIV 1/2	35 (66.0)	9 (17.0)	9 (17.0)	17 (32.1)	44 (83.0)	33 (62.3)	8 (15.1)	0 (0.0)	7 (13.2)	2 (3.8)	9 (17.0)	27 (50.9)	8 (15.1)
Immunoblot, GEENIUS HIV 1/2	33 (62.3)	12 (22.6)	8 (15.1)	33 (62.3)	45 (84.9)	5 (9.4)	0 (0.0)	0 (0.0)	6 (11.3)	2 (3.8)	12 (22.6)	33 (62.3)	0 (0.0)
EIA-RI index for Fiebig's stages defined with WB: Median (Ranges)									0.01 (0.01-0.04)	0.01 (0.01-0.01)	0.02 (0.01-0.19)	0.99	0.63 (0.16-0.99)

EIA-RI: Enzyme immunoassay for identification of recent HIV-1 infections, threshold to discriminate between < or > 180 days: 0.50.

6   *Table 2: Description of analyses for patients with high EIA-RI index*

Fiebig's Stages defined according to each confirmation assay	Preinclusion		Inclusion										
	p24 antigen	Confirmation test	Delay from preinclusion	CD4 T cell count (/mm³)	CD4/CD8 ratio	HIV-RNA (log copies/ mL)	HIV-DNA (log copies/ 10*6 PBMCs)	Antibodies, DUO ULTRA VIDAS	p24 antigen, DUO ULTRA VIDAS	WB, VIH1	INNO-LIA	Geenius	EIA-RI index
WB: V INNO-LIA: V Geenius: V	nr	INNO-LIA: gp120, gp41, P24	22	247	0.2	4.7	3.57	21.08 Positive	Negative	Positive gp160, gp110, p68, p55, gp41, p24+, p18±	Positive gp120, gp41, p24	Positive gp160, p24±, gp41	0.99
WB: VI INNO-LIA: VI Geenius: V	Positive	Geenius: gp160, gp41	14	602	1.5	1.53	1.0	13.71 Positive	Negative	Positive complete	Positive gp120, gp41, p31, p24, p17	Positive gp160, p24, gp41	0.83
WB: VI INNO-LIA: VI Geenius: V	Positive	Geenius: gp160, gp41	13	nr	nr	4.85	3.09	10.03 Positive	Negative	Positive gp160, p68, p55, gp41, p31, p24,	Positive gp120, gp41, p31, p24±	Positive gp160, gp41	0.99
WB: VI INNO-LIA: VI Geenius: V	nr	Geenius: gp160, gp41	13	307	0.6	6.09	3.41	13.92 Positive	Negative	Positive gp160, gp110, p68, p55±, p31, p24±, p18	Positive gp120, gp41, p31, p24±	Positive gp160, gp41	0.99
WB: VI INNO-LIA: V Geenius: V	nr	Geenius: gp160, gp41, p24	15	451	0.3	5.57	3.21	8.36 Positive	Negative	Positive gp160, p68, p55, gp41, p40, p31, p24, p18	Positive gp120, gp41, p24, p17	Positive gp160, p24, gp41	0.44
nr: not realized. WB: Western blot. Antibodies, DUO ULTRA VIDAS: relative fluorescence value (RVF) of antibodies ≥ 0.25: positive. EIA-RI: Enzyme immunoassay for identification of recent HIV-1 infections, threshold to discriminate between < or > 180 days: 0.50.													

7