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1 Title: Sensitivity and specificity of the new Bio-Rad HIV screening test, Access HIV combo V2

2

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32 Running title: Sensitivity and specificity of the Access HIV combo V2

33 Abstract (250/250 mots max):

34

35 **Background:** Diagnosing of Human Immunodeficiency Virus (HIV) types 1 and 2 requires a screening
36 with a highly sensitive and specific enzyme immunoassay and a low detection limit for the HIV-1 p24
37 antigen to minimize the diagnostic window.

38 **Objectives:** To determine the sensitivity, specificity and p24 limit of detection of the Access HIV
39 combo V2 assay.

40 **Study design:** Retrospective part of sensitivity: 452 HIV-1 positive samples from 403 chronic (9
41 different HIV-1 group M subtypes, 22 different HIV-1 group M CRFs, 3 HIV-1 group O), 49 primary
42 HIV-1 infections, 103 HIV-2 positive samples assessed at Pitié-Salpêtrière Hospital, 600 untyped HIV-
43 1, 10 subtype-D and 159 untyped HIV-2 samples assessed in Bio-Rad Laboratories. Prospective part of
44 clinical specificity: all consecutive samples in two blood donor facilities and Pitié-Salpêtrière (6570
45 patients) tested with Access HIV combo V2 and respectively Prism HIV O Plus (Abbott) or Architect
46 HIV Ag/Ab Combo (Abbott) for Ag/Ab screening, and Procleix Ultrio (Gen Probe) for HIV RNA
47 screening. Limit of detection of p24 antigen was assessed on recombinant virus-like-particles (10 HIV-
48 1 group M subtypes/CRFs, HIV-1 group O).

49 **Results:** Sensitivity (95% CI) of Access HIV combo V2 was 100% (99.63-100) for HIV-1 chronic
50 infection, 100% (98.55-100) for HIV-2 chronic infection and 100% (93.00-100) for HIV-1 primary
51 infection. Specificity (95% CI) was 99.98 (99.91-100). Limit of detection for p24 antigen was around
52 0.43 (IQR [0.38-0.56]) IU/mL, and consistent across the 11 analyzed subtypes/CRFs.

53 **Conclusions:** with both high sensitivity and specificity, Access HIV combo V2 is a suitable screening
54 assay for HIV-1/2 infection.

55

56

57 Keywords: Access HIV combo V2, HIV, sensitivity, specificity, accuracy, serology

59 Introduction

60 The first target to achieve the 2025 World Health Organization target of ending the Human
61 Immunodeficiency Virus (HIV) pandemic is that at least 95% of people living with HIV knows their
62 status [1]. HIV diagnosis faces three main challenges. Firstly, it has to detect antibodies directed to a
63 remarkable range of diverse antigens from both HIV-1 and HIV-2 [2,3]. Secondly, as a non-negligible
64 part of HIV transmission occurs early after an infection [4–6], diagnostic window should be as
65 reduced as possible. As a consequence, the assay must detect the HIV-1 specific p24 antigen at the
66 lowest limit of detection possible. Lastly, as a false positive HIV diagnosis can have deleterious
67 consequences, a high specificity is needed [7–9].

68 HIV diagnosis tests have remarkably improved since the beginning of the HIV pandemic, with current
69 recommended ones, or 4th generation assays, able to detect with high sensitivity and specificity all
70 circulating variants with a diagnostic window of about 2 weeks after infection [1,10–12].

71 The objective of this study was to assess sensitivity and specificity of Bio-Rad's new 4th generation
72 HIV test, Access HIV combo V2 on both real-life settings and well characterized commercial panels.
73 Additionally, we aimed to establish its limit of detection for the p24 antigen using diverse, well-
74 characterized commercial panels.

75 Materials and methods

76 Study design – sample collection.

77 This study included a multicenter retrospective part of clinical sensitivity and a multicenter
78 retrospective and prospective part of clinical specificity.

79 Two sites were involved in clinical sensitivity. The Service of Virology at the Pitié-Salpêtrière Hospital
80 (Paris, France) provided 452 HIV-1 positive samples from 403 chronic and 49 primary HIV-1 infected
81 patients, and 103 HIV-2 positive samples from chronically infected patients. HIV-1 serum samples
82 from chronically infected patients were selected to account for a large part of HIV-1 diversity (sup
83 table 1) : 9 different HIV-1 group M subtypes, 22 different HIV-1 group M CRFs, 3 HIV-1 group O.
84 Serum samples from HIV-1 primary infection, of which 9 were positive for p24-antigen only (Stage II
85 and III Fiebig [13]), were mostly of HIV-1 subtype B, CRF02 and CRF06 (sup table 1). HIV-antibody and
86 p24 positivity were assessed using Architect HIV Ag/Ab Combo (Abbott®, Rungis, France) and Liaison
87 XL HIV Ab/Ag (Diasorin, Antony, France) as screening assays, with New LAV Blot I and II (Bio-Rad
88 laboratories, Marnes-la-Coquette, France) as confirmatory and differentiation assays. Subtypes and
89 recombinant forms of HIV-1 strains were determined using molecular assay as previously described
90 [14]. Samples were stored frozen at -20°C until use. HIV-positive samples tested at the Bio-Rad
91 Laboratories originated from commercial panels supplied by Seracare, Zeptometrix, and Biomex for
92 seroconversion panels, as detailed in Sup Table 2. Days to first reactive results were compared with
93 the Architect assay. For the Architect assay, we used seroconversion panel data provided by the
94 manufacturer, followed if unavailable by the FDA's published data. If this data was still missing, we
95 extended our review to previously published studies. Chronic HIV samples tested in Bio-Rad
96 Laboratories included a total of 600 ungenotyped HIV-1, 10 HIV-1 subtype D samples and 159
97 ungenotyped HIV-2 samples.

98 For the retrospective part of clinical specificity, 203 frozen serum samples from HIV-negative
99 pregnant women who consulted at Pitié-Salpêtrière Hospital from April 2019 to January 2020 and 10

100 HTLV+/HIV- serum samples were analyzed. HIV status was assessed using Architect HIV Ag/Ab
101 Combo.

102 For the prospective part of clinical specificity were included: fresh serum or plasma samples from all
103 consecutive patients with an HIV testing at Pitié-Salpêtrière Hospital from 30/01/2020 to 17/03/2020
104 (1509 samples), all samples from consecutive blood donors at the Etablissement Français du Sang
105 (EFS) of Bois-Guillaume (France) from 28/01/2020 to 11/02/2020 (2512 samples), and all consecutive
106 blood donors from the EFS of Lille (France) from 10/02/2020 to 13/03/2020 (2549 samples).

107 [Access HIV combo V2 assay](#)

108 The Access HIV combo V2 assay is a paramagnetic-particle, semi-quantitative chemiluminescent
109 immunoassay (CLIA) designed to detect HIV-1 p24 and HIV-2 p26 antigens, and antibodies to HIV-1
110 and HIV-2 in human serum or plasma. The test is configured to run on Beckman Coulter's Access
111 immunoassay systems, with a run time of 30 minutes. Results are expressed as S/CO ratio with S/CO
112 <0.9 considered as non-reactive, S/CO ≥1 reactive and S/CO [0.9-<1] gray zone. This test does not
113 distinguish antigen from antibody reactivity.

114 [Sample processing](#)

115 For the retrospective parts of sensitivity and specificity, all samples were tested with the Access HIV
116 combo V2 according to the manufacturer's recommendations. Test found negative for the study of
117 sensitivity were planned to be repeated once.

118 For the prospective part of specificity, all samples were tested in parallel with Access HIV combo V2
119 and either Architect HIV Ag/Ab Combo at the Pitié-Salpêtrière Hospital, or Prism HIV O Plus (Abbott)
120 and Procleix Ultrio (Novartis Diagnostics, Emeryville, CA, USA) at the EFS centers. All gray zone and
121 reactive samples were retested in duplicate, followed, if remaining gray zone or reactive, by a
122 confirmatory assay. Confirmatory assays were conducted using either New LAV Blot I and II at Pitié
123 Salpêtrière Hospital, or INNO-LIA HIV I/II Score (Innogenetics, Gent, Belgium) at the EFS centers.

124 [P24 and p26 antigens analytical sensitivity](#)

125 Access HIV combo V2 assay sensitivity for p24 and p26 antigens was assessed on the Access 2
126 platforms using the NIBSC/WHO p24 antigen standards (NIBSC 90/636 and 16/210) and p26 antigen
127 (NIBSC 16/236) with the following dilutions: 1:2, 1:4, 1:8, 1:16, 1:32. Both antigens were diluted in
128 sterile water.

129 [Statistical analysis](#)

130 Statistical analysis was conducted using R version 4.2.1 software [15]. Categorical variables were
131 expressed as numbers (percentages) and continuous variables as medians (interquartile ranges
132 [IQR]). The 95% confidence intervals (95% CI) were calculated using Wilson confidence interval for
133 proportions [16]. Limit of detection was assessed for p24 antigen using linear regression to calculate
134 the amount of p24 detected for a S/CO of 1. Comparison was done using Chi-squared test for
135 categorical variables, with a significance assigned at a p value < 0.05. Sample size was determined
136 according to the European Commission decision on technical specifications for in vitro diagnostic
137 medical devices [17].

138 [Role of the study sponsor](#)

139 The sponsor provided reagents and automated equipment used in this study and was responsible for
140 data collection. First and last authors had full access to the study database, generated statistical
141 analyses, prepared first draft of the manuscript and made the decision to submit the manuscript for

142 publication. Y.C, D.N and M.C are employed by Bio-Rad. V.G, A.GD, C.D, S.G received no personal
143 funding from the study sponsor.

144 Ethics

145 The study was conducted according to the principles of the Declaration of Helsinki and in conformity
146 with institutional regulations and guidelines. The evaluated method was performed on sample
147 excess. Patients were systematically notified of any supplementary biological analyses on frozen
148 samples, initially collected as part of routine clinical practice.

149

150 Results

151 Sensitivity on chronic HIV infection

152 All the 403 HIV-1 samples from the retrospective study collected at the Pitié-Salpêtrière Hospital as
153 well as the 25 HIV-1 samples collected during the prospective study of specificity were reactive,
154 yielding a sensitivity of 100% (95%CI [99.11-100]). Each of the 600 ungenotyped and 10 HIV-1
155 subtype D samples tested in Bio-Rad Laboratories were also reactive, owing a 100% (95% CI [99.37-
156 100]) sensitivity in Bio-Rad Laboratory.

157 All the 103 retrospective chronically HIV-2 samples collected at Pitié-Salpêtrière Hospital as well as all
158 the 159 HIV-2 samples at Bio-Rad Laboratories were positive, owing a sensitivity of 100% (95% CI
159 [96.40-100]) at Pitié-Salpêtrière and 100% (95% CI [97.64-100]) in Bio-Rad Laboratories.

160 Pooled estimated sensitivity for the diagnosis of chronic HIV-1 infection was 100% (95% CI [99.63-
161 100]) and for the diagnosis of chronic HIV-2 infection at 100% (95% CI [98.55-100]). These results are
162 summarized fig 1, and S/CO values reported in sup fig 1.

163 Sensitivity for HIV-1 primary infection

164 All the 49 retrospective and 2 prospective samples from primary HIV-1 infection collected at Pitié-
165 Salpêtrière Hospital were found reactive on Access HIV combo V2, yielding a sensitivity of 100% (95%
166 CI [93.00-100]). Corresponding S/CO values are reported in sup fig 1.

167 A total of 415 samples from 41 commercial seroconversion panels were tested in Bio-Rad
168 Laboratories. Results aligned closely with Architect (reference assay). Day to first reactive result was
169 identical for 35 (85.4%) of them, while Access outperformed for 5 (12.2%, with respectively 5, 6, 7, 3
170 and 5 days later for the Architect) and underperformed for 1 (2.4%, with 4 days earlier for the
171 Architect) of them (sup table 2). Discrepant results between the two assays are summarized table 1.

172 Specificity

173 Blood donor samples, routinely tested at EFS Hauts de France–Normandie in Lille (n=2549) and Bois-
174 Guillaume (n=2512) with *Prism HIV O plus* and *Procleix Ultrio* were prospectively analyzed in parallel
175 with Access HIV combo V2. No initial false-reactive sample was identified at the first site, while at the
176 second site, 4 samples were found initial false-reactive (IR). These 4 IR samples were negative after
177 repeating in duplicate. This resulted in an overall IR specificity for blood donors of 99.92% (95% CI
178 [99.80-99.97]) and an overall specificity after repeat testing of 100% (95% CI [99.92-100]). In
179 comparison, Prism HIV O plus assay gave 4 RR false-reactive samples on the blood donor's
180 population. There was no false-reactive sample identified with the Nucleic Acid Amplification Test
181 (NAAT) *Procleix Ultrio*, owing a specificity at 100% (95% CI [99.92-100]). Of note, no sample was
182 identified as true reactive.

183 At Pitié-Salpêtrière Hospital, among the 1509 samples tested prospectively in parallel with Architect,
184 27 (1.8%) were found to be true reactive samples (25 chronic and 2 primary infections), while 5 were
185 IR and 1 was repeatedly false-reactive. As so, the hospitalized patient IR specificity assessed at was
186 99.66% (95% CI [99.74-99.83]) and RR specificity 99.93% (95% CI [99.62-99.99]). Architect specificity
187 was identical, with 2 other repeatedly false-reactive samples. We also performed a retrospective
188 exploratory analysis on 203 hospitalized HIV-negative pregnant women. There was no false-reactive
189 sample, owing a specificity of 100% (95% CI [98.1-100]). Of note, RR hospitalized patient specificity
190 was not statistically significantly lower than blood donor specificity ($p = 0.51$). Also, we performed an
191 exploratory analysis on 10 HTLV-1 positive / HIV-negative samples. One sample tested reactive, with
192 an S/CO ratio at 1.46, repeated at 1.86 and 1.81. Architect was also reactive on this sample. As the
193 HIV Western blots were negative, this sample was considered as a false reactive sample.

194 As so, pooled (blood donors and hospitalized patients) IR specificity was 99.86% (95% CI [99.74-
195 99.83]) and RR specificity was 99.98% (95% CI [99.91-100%]). Specificity results are summarized fig 2
196 and S/CO distribution for negative results summarized sup fig 1.

197 Analytical p24 and p26 antigens sensitivity

198 The limits of detection for p24 and p26 were assessed using WHO standardized panels (Table 2). The
199 1st international reference sample for HIV-1 Subtype B had a detection limit of 0.39 IU/mL. The limit
200 of detection for the p24 antigen was consistent across the 11 HIV-1 subtypes, ranging from 0.27 to
201 0.58 IU/mL with a median of 0.43 (IQR [0.38-0.56]). Since p26 had no assigned unitage, its limit of
202 detection was determined using serial dilution, with samples yielding positive results up to a dilution
203 of 1/8.

204 Discussion

205 Overall, Access HIV combo V2 displayed high sensitivity for both chronic HIV-1 samples with a
206 sensitivity of 100% (95% CI [99.63-100]), and for HIV-1 primary infection samples with a sensitivity of
207 100% (95%CI [93.00-100]). The sensitivity for HIV-2 infection was also 100% (95% CI [98.55-100]).
208 Specificity was also high, at 99.98% (95% CI [99.91-100]). Limit of detection of p24 antigen was low,
209 around 0.43 IU/mL and consistent across the analyzed HIV-1 groups, subtypes/CRFs.

210 High sensitivity and specificity were expected findings, consistent with previous reports from all
211 other commercial 4th generation assays [11,18–23]. Analytical sensitivity for p24 antigen was around
212 0.43 IU/mL, consistent across the 11 HIV-1 subtypes/CRFs. This finding contrasts with the previous
213 Access HIV combo version, which had a limit of detection for subtype B around 3 times higher [24]
214 and performed very poorly on non-subtype B samples, with limit of detection often over 10 IU/mL
215 [25]. Compared with published data [26], Access HIV combo V2 (median, [IQR] p24 Ag limit of
216 detection of 0.43 IU/mL, [0.38-0.56 IU/mL]) had a similar limit of detection as ARCHITECT HIV Ag/Ab
217 Combo (0.57 IU/mL, [0.43-0.64 IU/mL]) and BioPlex 2200 HIV Ag-Ab assays (0.27 IU/mL [0.21-0.36]),
218 outperformed Liaison[®] XL Murex HIV ab/Ag and Elecsys[®] HIV combi PT assays (0.67 IU/mL, [0.58-
219 0.72]), but underperformed if compared with the Elecsys HIV Duo assay (0.33 IU/mL, [0.30-0.37
220 IU/mL]), as described in sup table 3. However, we were unable to link these gaps in detection
221 thresholds to potential difference in HIV-1 window period, as to date we have not managed to gather
222 any non-reactive 4th generation HIV-1 sample that was positive on HIV-1 NAAT. As this contrast with
223 current guidelines that advise the use of NAAT in this setting [27,28], further studies are needed to
224 address the relevance of this guideline in the setting of increasing p24 sensitivities of 4th generation
225 assays. This study has several limitations. The genotypes of HIV-1 responsible for primary infection at
226 Pitié-Salpêtrière were predominantly HIV-1 group M subtype B and CRF02_AG, reflecting the French
227 and European epidemiologies [2]. Furthermore, since their sera were primarily screened using the

228 Architect platform, this part of the study couldn't ascertain if Access had a shorter window period.
229 Regarding the commercial seroconversion panel, a similar bias toward subtype B might exist since
230 most blood samples originate from US patients. Furthermore, Architect's results were extracted from
231 manufacturer's data or previously published studies (Sup table 2) instead of being generated from
232 samples stored within the same conditions. The limits of detection for p24 antigen from different
233 HIV-1 subtypes and p26 antigen were derived from commercial recombinant virus-like particles
234 rather than patient sera, to facilitate future comparison. This specification, however, represents a
235 surrogate marker for HIV-1 primary infection and cannot be rigorously translated into window
236 periods. Finally, this assay is designed to detect p26 antigen, to shorter the window period for HIV-2
237 infection. However, due to a lack of HIV-2 primary infection sample we were unable to validate this
238 hypothesis.

239 As a conclusion, Access HIV combo V2, with both high sensitivity and specificity is a suitable
240 screening assay for HIV-1 and HIV-2 infections.

241

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345 Table 1: Commercial seroconversion panels with conflicting results between Access HIV combo V2
 346 and Abbott's Architect.

| Vendor | Sample ID | Days to first reactive result | | Difference of first reactive result (Days)* | Source for Architect results |
|----------------|-----------|-------------------------------|-----------|---|------------------------------|
| | | Access Combo V2 | Architect | | |
| Seracare / BBI | PRB944 | 2 | 7 | 5 | FDA notice |
| | PRB945 | 7 | 13 | 6 | T. Sano et al [23] |
| | PRB953 | 7 | 3 | -4 | Manufacturer |
| | PRB957 | 16 | 23 | 7 | FDA notice |
| | SC9018 | 25 | 28 | 3 | Manufacturer |
| | SC12008 | 23 | 28 | 5 | Manufacturer |

347 * a positive number indicates that Access Combo V2 is reactive before Architect, while a negative
 348 result indicates that Architect is reactive before Access Combo V2.

349

350 Table 2: Analytical sensitivity of the Access HIV
351 combo V2 assay on the Access platform for HIV-
352 1 p24 antigen according to group, subtypes and
353 CRFs

| HIV-1 Subtype | Analytical Sensitivity (IU/mL) |
|--------------------------------|--------------------------------|
| B ^a | 0.39 |
| A1 ^b | 0.56 |
| B ^b (16/214) | 0.27 |
| B ^b (16/216) | 0.35 |
| C ^b | 0.47 |
| D ^b | 0.53 |
| F1/CRF12_BF/BFrec ^b | 0.43 |
| G ^b | 0.56 |
| CRF20_BG ^b | 0.38 |
| CRF01_AE ^b | 0.56 |
| CRF02_AG ^b | 0.36 |
| H ^b | 0.42 |
| Group O ^b | 0.58 |

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355 ^a WHO reference panel 90/636

356 ^b WHO reference panel 16/210

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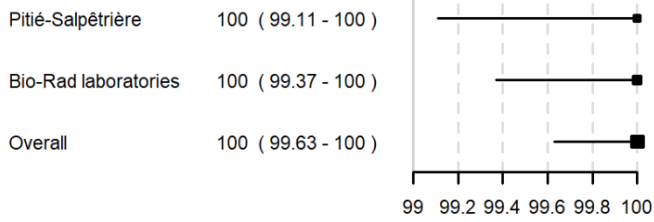
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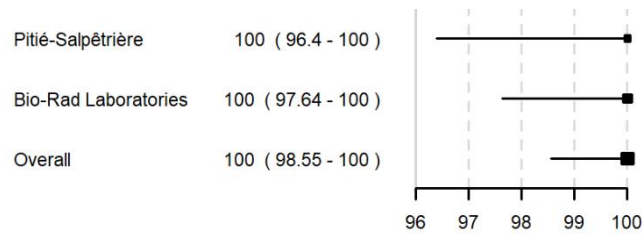
366

367

A



B



368 Figure 1: Sensitivity of the Access HIV combo V2 for HIV-1

369 (A) and HIV-2 (B) chronic infection

370

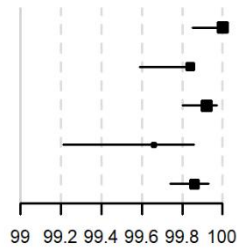
371

372

A

373

| | |
|--------------------|-------------------------|
| EFS Lille | 100 (99.85 - 100) |
| EFS Bois-Guillaume | 99.84 (99.59 - 99.84) |
| EFS Overall | 99.92 (99.8 - 99.97) |
| Pitié-Salpêtrière | 99.66 (99.21 - 99.86) |
| Overall | 99.86 (99.74 - 99.93) |

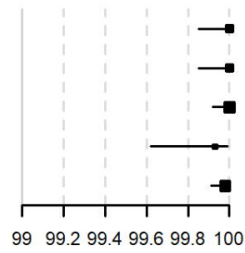


377

B

378

| | |
|--------------------|-------------------------|
| EFS Lille | 100 (99.85 - 100) |
| EFS Bois-Guillaume | 100 (99.85 - 100) |
| EFS Overall | 100 (99.92 - 100) |
| Pitié-Salpêtrière | 99.93 (99.62 - 99.99) |
| Overall | 99.98 (99.91 - 100) |



382

383

384 Figure 2: Specificity of the Access HIV combo V2 on first result (A) and after repeat (B)

385

386

387

388 Supplementary table 1: Subtypes and recombinant forms of retrospective HIV-1 samples used at
 389 Pitié-Salpêtrière Hospital.

| Subtype/ group/CRF | Stage | No Specimens |
|--------------------------|---------|--------------|
| Subtype A | Chronic | 34 |
| Subtype B | Chronic | 94 |
| Subtype C | Chronic | 20 |
| Subtype D | Chronic | 14 |
| Subtype F | Chronic | 17 |
| Subtype G | Chronic | 29 |
| Subtype H | Chronic | 6 |
| Subtype J | Chronic | 1 |
| Subtype K | Chronic | 2 |
| Group O | Chronic | 3 |
| CRF01 | Chronic | 22 |
| CRF02 | Chronic | 97 |
| CRF06 | Chronic | 15 |
| CRF08 | Chronic | 1 |
| CRF09 | Chronic | 7 |
| CRF10 | Chronic | 1 |
| CRF11 | Chronic | 8 |
| CRF13 | Chronic | 6 |
| CRF14 | Chronic | 6 |
| CRF15 | Chronic | 2 |
| CRF18 | Chronic | 3 |
| CRF19 | Chronic | 2 |
| CRF20 | Chronic | 1 |
| CRF22 | Chronic | 2 |
| CRF25 | Chronic | 1 |
| CRF30 | Chronic | 1 |
| CRF36 | Chronic | 2 |
| CRF37 | Chronic | 1 |
| CRF42 | Chronic | 1 |
| CRF44 | Chronic | 1 |
| CRF45 | Chronic | 1 |
| CRF60 | Chronic | 2 |
| Subtype A | Primary | 1 |
| Subtype B | Primary | 20 |
| Subtype C | Primary | 2 |
| Subtype D | Primary | 1 |
| CRF01/CRF15 ^a | Primary | 1 |
| CRF02 | Primary | 10 |
| CRF06 | Primary | 4 |
| CRF18 | Primary | 2 |
| Unknown | Primary | 8 |

390

391 ^a Genotyping was unable to distinguish between CRF01 and CRF15

392

393 Supplementary table 2: Commercial seroconversion panels used for the present study with results
 394 for Access HIV combo V2 and Abbott's Architect.

| Vendor | Sample ID | Days to first reactive result | | Source for Architect results |
|----------------|--------------|-------------------------------|-----------|------------------------------|
| | | Access Combo V2 | Architect | |
| Seracare / BBI | PRB944 | 2 | 7 | FDA notice [1] |
| | PRB945 | 7 | 13 | T. Sano et al [2] |
| | PRB949 | 18 | 18 | Manufacturer |
| | PRB950 | 18 | 18 | Manufacturer |
| | PRB953 | 7 | 3 | Manufacturer |
| | PRB954 | 17 | 17 | Manufacturer |
| | PRB955 | 3 | 3 | Manufacturer |
| | PRB957 | 16 | 23 | FDA notice [1] |
| | PRB958 | 7 | 7 | FDA notice [1] |
| | PRB964 | 22 | 22 | Manufacturer |
| | PRB966 | 44 | 44 | Manufacturer |
| | PRB969 | 63 | 63 | Manufacturer |
| | PRB970 | 0 | 0 | Manufacturer |
| | PRB973 | 7 | 7 | Manufacturer |
| | PRB975 | 14 | 14 | Manufacturer |
| | SC-0600-0270 | 30 | 30 | Manufacturer |
| | SC-0600-0271 | 7 | 7 | Manufacturer |
| | SC-0600-0272 | 18 | 18 | Manufacturer |
| Zeptomatrix | SC9011 | 36 | 36 | Manufacturer |
| | SC9012 | 16 | 16 | Manufacturer |
| | SC9013 | 25 | 25 | Manufacturer |
| | SC9016 | 30 | 30 | Manufacturer |
| | SC9018 | 25 | 28 | Manufacturer |
| | SC9020 | 90 | 90 | Manufacturer |
| | SC9021 | 47 | 47 | Manufacturer |
| | SC9023 | 78 | 78 | Manufacturer |
| | SC9024 | 53 | 53 | Manufacturer |
| | SC9025 | 85 | 85 | Manufacturer |
| | SC9026 | 44 | 44 | Manufacturer |
| | SC9030 | 47 | 47 | Manufacturer |
| | SC9031 | 146 | 146 | Manufacturer |
| | SC9033 | 82 | 82 | Manufacturer |
| | SC9089 | 16 | 16 | Manufacturer |
| | SC6244 | 28 | 28 | Manufacturer |
| | SC12008 | 23 | 28 | Manufacturer |
| Biomex | SCP-HIV-002 | 63 | 63 | Manufacturer |
| | SCP-HIV-003 | 17 | 17 | Manufacturer |
| | SCP-HIV-004 | 56 | 56 | Manufacturer |
| | SCP-HIV-005 | 16 | 16 | Manufacturer |
| | SCP-HIV-006 | 15 | 15 | Manufacturer |
| | SCP-HIV-007 | 12 | 12 | Manufacturer |

395

396

397 Supplementary table 3: Summary of the p24 antigen limit of detection (IU/mL) on the WHO panel for
 398 six 4th generation assays. Data for comparative assays were extracted from Qiu et al. [3].

399

| | Access HIV combo V2 | ARCHITECT HIV Ag/Ab Combo | Liaison® XL murex HIV ab/Ag HT | Elecsys HIV Duo | Elecsys® HIV combi PT | BioPlex 2200 HIV Ag-Ab |
|--|------------------------|------------------------------|-----------------------------------|---------------------|--------------------------|---------------------------|
| Median (IQR) ^{1,2} | 0.43 (0.38-0.56) | 0.57 (0.43-0.64) | 0.67 (0.58-0.72) | 0.33 (0.30-0.37) | 0.89 (0.74-1.04) | 0.27 (0.21-0.36) |
| P-value for comparison with Access ³ | NA ⁴ | 0.24 | 0.0012 | 0.02 | 0.0005 | 0.13 |

400 1: Inter Quartile Range

401 2: Results are expressed as IU/mL

402 3: Based on Wilcoxon's test for paired samples

403 4: Not applicable

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413 of acute HIV infection, *Journal of Clinical Virology.* 145 (2021) 105022.
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