

1 **Whatman FTA[®] cards versus plasma specimens for the quantitation of HIV-1**
2 **RNA using two Real-Time PCR assays**

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22

23 **Abstract**

24 **Background:** Several studies have been conducted to compare the use DBS as
25 alternative to plasma specimens, but mainly using Whatman 903[®] cards as filter
26 paper. The aim of this study was to evaluate Whatman FTA[®] cards (FTA cards)
27 specimens for HIV-1 viral load testing by comparing it to plasma specimens, using 2
28 real-Time PCR assays.

29 **Methodology:** A cross-sectional study was conducted between April 2017 and
30 September 2017, in HIV-1 patients admitted at Yalgado Ouédraogo teaching
31 hospital. Paired FTA cards and plasma specimens were collected and analyzed
32 using Abbott RealTime HIV-1 assay (Abbott) and COBAS[®] AmpliPrep/COBAS[®]
33 TaqMan v2.0 (Roche), following manufacturers' protocol.

34 **Results:** A total of 107 patients were included. No Statistical differences (p-value >
35 0.05) were observed between the mean viral loads obtained from FTA cards and
36 plasma specimens with Roche and Abbott assays. Twenty-nine samples with Roche
37 and 15 samples with Abbott assay showed discrepant results. At viral loads of ≤ 1000
38 copies/mL, the sensitivity and specificity of FTA cards were 78.6%, and 100% with
39 Roche, and 92.3% and 95.9% with Abbott. Strong correlation was found between
40 FTA cards and plasma specimens with both assays. With Roche, Bland-Altman
41 analysis showed bias of -0.3 and 95% limits of agreement of -2.6 to 1.8 log₁₀, with
42 97/99 cases (97.9%) within agreement limits. With Abbott, Bland-Altman analysis
43 showed bias of -0.1 and 95% limits of agreement of -2.3 to 2.1 log₁₀, with 96/99
44 cases (96.9%) within agreement limits.

45 **Conclusion:** Our study demonstrated the feasibility of using FTA cards filter paper
46 for HIV-1 viral load testing. However, further studies are required for FTA cards filter
47 paper validation in HIV-1 treatment monitoring.

48

49 **Keywords:** Whatman FTA[®] cards, dried blood spots, HIV-1, viral load, real-time
50 PCR, Burkina Faso.

51

52

53 **Introduction**

54 Viral load (VL) testing is the gold standard for HIV treatment monitoring. Periodic VL
55 tests are the most accurate way of determining whether antiretroviral therapy (ART)
56 is working to suppress replication of the virus [1–3]. With ART rapidly expanding in
57 resource-limited settings, VL is a fundamental and even crucial issue in scaling up
58 antiretroviral treatment. However, many barriers exist to VL testing in resource-limited
59 settings, including lack of basic essential equipment, storage and transport limitations
60 for whole blood and plasma[4]. Due to the lability of viral RNA, whole blood in
61 EDTA/K3 tubes cannot be stored more than 6 hours at 25°C[5,6]. Plasma storage
62 and transport require that plasma is transported within 24 hours at 25°C in EDTA/K3
63 tubes, or within 5 days at 4°C for EDTA/K3 tubes, after centrifugation [7]. In low and
64 middle-income countries, such restrictive guidance on whole blood and plasma
65 transport greatly limits access to VL testing to only those in close proximity to national
66 or regional laboratories. Therefore, a simple method is needed to allow access to
67 HIV-1 VL testing for patients in rural areas.

68 Since June 2013, WHO has been recommending the use of dried blood spot (DBS)
69 as alternative to plasma for collection, transport, and HIV-1 VL testing and
70 genotyping drug resistance [8,9]. DBS are an inexpensive and practical alternative to
71 plasma; samples are easy to transport, without the need for cold chains or complex
72 equipment; a further benefit of DBS is the reduction in blood sample volume [10,11].

73 Numerous studies carried out in Burkina Faso and other countries have shown a
74 strong correlation between DBS and plasma specimens for HIV-1 VL testing [12–16].
75 However, in most of these studies, Whatman 903[®] cards filter paper was the only
76 filter paper that has been used for HIV-1 load [17]. Diversifying the type of filter
77 papers available for HIV-1 treatment monitoring could reduce shortages risk,
78 decrease costs through price competition and increase the availability of filter paper.
79 In Burkina Faso, another type of paper is now also routinely used for sample
80 collection during malaria vigilance programs and antimalarial drug trials: Whatman
81 FTA[®] cards filter paper.

82 The aim of this study was to evaluate Whatman FTA[®] Cards (FTA cards) specimens
83 for HIV-1 VL testing by comparing it to plasma specimens, using COBAS[®]
84 AmpliPrep/COBAS[®] TaqMan v2.0 HIV-1 test (Roche) and Abbott RealTime HIV-1
85 assay (Abbott).

86 **Methodology**

87 **Study site and design**

88 A cross-sectional study was conducted between April and September 2017, at the
89 National Reference Laboratory for HIV/AIDS and Sexually Transmitted Infections,
90 located at Yalgado Ouédraogo teaching hospital (CHU-YO), 03 BP 7022, Ouaga 03,
91 Ouagadougou, Burkina Faso. Socio-demographic, clinical, and laboratory data were
92 obtained from the study subjects using a structured questionnaire and laboratory
93 analysis blood samples.

94

95 **Study population**

96 HIV-1 patients admitted at CHU-YO were the study population. The inclusions criteria
97 were: Patients infected with HIV-1, who consented, antiretroviral-naive patients or
98 patients under antiretroviral treatment.

99

100 **Sample collection and processing**

101 Venous collection of whole blood was performed in 2 EDTA/K3 tubes of 4.7 mL or a
102 single EDTA/K3 tube of 10 mL from patients during their routine visits to the CHU-
103 YO. Before plasma separation, DBS were prepared by dispensing 50 μ L and 100 μ L
104 of blood per spot (2 spots per card) onto FTA cards and dried at room temperature
105 (25 ± 2 °C) for 18-24 hours. The FTA cards were stored in zip-lock plastic bags with 2
106 silica gel desiccants at room temperature upon receipt. Plasma was prepared by
107 centrifugation of the whole blood, aliquoted, and stored at -70 °C until testing for
108 HIV-1 viral load. FTA cards samples were analyzed not more than 14 days after
109 being deposited. Processing of FTA cards for elution of RNA was done as per the
110 protocol for HIV-1 RNA quantitation provided by the ROCHE and ABBOTT
111 diagnostics to be used on their assays systems, COBAS[®] AmpliPrep/COBAS[®]
112 TaqMan v2.0 HIV-1 test and m2000rt, respectively.

113 .

114 **Viral Load Quantification**

115 VL was measured from the paired FTA cards and in plasma specimens with Roche
116 and Abbott assays, following manufacturers' protocol. VL results obtained from FTA
117 cards specimens were then compared with that of plasma specimens (gold
118 standard).

119 **Statistical analysis**

120 The statistical analyzes were performed using RStudio (Version 0.99.903).
121 Sensitivity, specificity predictive positive value and predictive negative value were
122 estimated to determine the performances of FTA cards for quantification of HIV-1 VL
123 at a viral load threshold of 1000 copies/ml, a decision point for therapeutic efficacy.
124 Bland–Altman analysis was used to measure agreement in viral load values obtained
125 from FTA cards and plasma specimens. Correlations between viral loads obtained
126 from FTA cards and plasma specimens were assessed with the Pearson statistical
127 test. All HIV-1 VL values were log₁₀ transformed prior Bland–Altman and correlation
128 analysis. The significance level was set at a p-value of 0.05.

129

130 **Ethical considerations**

131 An informed consent form was presented to each patient prior to blood collection and
132 patients who gave verbal consent were enrolled. Additionally, in order to guarantee
133 confidentiality, random anonymous identification numbers were assigned to each
134 patient.

135 **Results**

136 **Patient characteristics**

137 A total of 107 patients were included. The mean age of the patients was 42.0 ± 13.4
138 years (ranging from 1 days to 77 years). Most patients were female (sex ratio =0.39).

139

140 **Sample collection and bioanalysis**

141 Whole blood was collected from all the patients and the paired FTA cards and
142 plasma specimens collected were analyzed with Abbott and Roche assays for HIV-1
143 RNA VL. Of all 107 paired FTA cards and plasma specimens tested, 8 FTA cards
144 specimens gave an invalid result with the Abbott assay and were thus excluded from
145 further analysis. Thus, out of 107 paired FTA cards and plasma samples collected,
146 99 were analysis.

147

148

149 **Comparison between FTA cards and plasma specimens in HIV-1 RNA**
150 **quantitation**

151

152 ***With Roche assay***

153 No statistical differences (p-value = 0.1704) were observed between the mean VL
154 obtained from FTA cards (1.75 log₁₀) and plasma (1.37 log₁₀) specimens (**Figure**
155 **1A**).

156 A total of 29 samples showed discrepant results. Eight (17.0%) samples tested not
157 detected on FTA cards but were moderate positive (n=7; 14.9%) and high positive
158 (n=1; 2.1%) on plasma specimens. Twenty-one (70.0%) samples tested moderate
159 positive on FTA cards specimens but gave not detected (n=16, 53.3%) and high
160 positive (n=5; 16.7%) results on plasma specimens (**Table 1**).

161

162 ***With Abbott assay***

163 No statistical differences (p-value = 0.72) were observed between the mean VL
164 obtained from FTA cards (1.50 log₁₀) and plasma (1.38 log₁₀) specimens (**Figure**
165 **1B**).

166 A total of 15 samples showed discrepant results. Twelve (16.7%) samples tested not
167 detected on FTA cards specimens but were moderate positive (n=7; 14.9%) and high
168 positive (n=2; 2.8%) on plasma specimens. Three (11.1%) samples tested high
169 positive on FTA cards specimens but gave not detected (n=1; 3.7%) and moderate
170 positive (n=2; 7.4%) results on plasma specimens (**Table 2**).

171

172 **Performance of FTA cards for HIV-1 RNA quantitation**

173 ***With Roche assay***

174 The sensitivity and specificity of FTA cards at VL of ≤1000 copies/mL were 78.6%,
175 and 100%, respectively (**Table 3**).

176

177 ***With Abbott assay***

178 The sensitivity and specificity of FTA cards at VL of ≤1000 copies/mL were 92.3%
179 and 95.9%, respectively (**Table 3**).

180 **Correlation and agreement between FTA cards and plasma specimens in HIV-1**
181 **RNA quantitation**

182

183 ***With Roche assay***

184 There was a strong correlation ($R^2 = 0.790$; $p\text{-value} < 2.2e-16$) between FTA cards
185 and plasma specimens values (**Figure 2A**). Bland-Altman analysis showed bias of -
186 0.3 and 95% limits of agreement of -2.6 to 1.8 log₁₀; total number of cases within
187 agreement limits in this study was 97/99 (97.9%) (**Figure 3A**).

188

189 ***With Abbott assay***

190 The correlation between VL values obtained from FTA cards and plasma specimens
191 tested was strong ($R^2 = 0.764$; $p\text{-value} < 2.2e-16$) (**Figure 2B**). Bland-Altman analysis
192 showed bias of -0.1 and 95% limits of agreement of -2.3 to 2.1 log₁₀; total number of
193 cases within agreement limits in this study was 96/99 (96.9%) (**Figure 3B**).

194

195 **Discussion**

196 Several studies have been conducted to compare the use DBS as alternative to
197 plasma specimens, but mainly using Whatman 903[®] as filter paper [17]. In Burkina
198 Faso, another type of paper (FTA cards) is now also routinely used for sample
199 collection during malaria vigilance programs and antimalarial drug trials. In this study,
200 FTA cards was evaluated as an alternative sample collection method to plasma for
201 HIV-1 RNA quantitation using commercial Roche and Abbott assays. To the best of
202 our knowledge, this was the first study to evaluate and compare the use of FTA cards
203 filter paper (for DBS) to plasma specimens for VL testing using both Roche and
204 Abbott assays.

205 In our study, no statistical differences ($p\text{-value} > 0.05$) were observed between the
206 mean VL obtained from FTA cards and plasma specimens using Roche and Abbott
207 assay. These findings are similar to those of previous reports obtained using
208 Whatman 903[®] cards [18–20]. However, in this study, 17.0% samples tested not
209 detected on FTA cards, were positive on plasma specimens, with 2.1% high positive.
210 This observation of discrepant results is consistent with findings from other studies
211 using Whatman 903[®] cards [15,21–23]. The reasons of these discrepant results were
212 well documented in the literature[15,17,21–23]. In a systematic review published in

213 2014, Smit et al. [17] have indicated that the most important reasons DBS is not, and
214 may never be, as sensitive as plasma is because of the differences in sample volume
215 between DBS and plasma. In the current study, the sample volume used on FTA
216 cards was 50 μ L and 100 μ L, with the Roche and Abbott assays, respectively.
217 Hematocrit has been suggested to recalculate DBS VL to plasma VL copies/mL by
218 applying the difference between plasma and DBS sample volume [24,25]. To make
219 the calculation, hematocrit values can be obtained to adjust DBS VL results by
220 calculating the amount of plasma in a DBS sample. The current study did not use
221 hematocrit adjustment. Indeed, according to the manufacturer's protocol, a
222 hematocrit adjustment is not required for the calculation of VL obtained from DBS
223 with the Roche and Abbott assays.

224 An over estimation of HIV-1 RNA levels in FTA cards specimens with low-level
225 viremia (below 1000 copies/mL) was observed in this study; this overestimation was
226 also highlighted in the Bland-Altman analysis (means difference of -0.3 and -0.1
227 \log_{10} , with Roche and Abbott assays, respectively). This observation is consistent
228 with findings by others with DBS [9,17,19,21,23]. A possible and most advanced
229 explanation of this repeated finding could be the contribution of intracellular HIV-1
230 DNA and RNA which is present in the DBS but not in the plasma counterpart [26,27].
231 Vidya et al. [18] suggested that the contribution of intracellular HIV-1 DNA and RNA
232 could be more relevant to specimens with low or undetectable levels viremia than to
233 specimens containing higher levels of extracellular HIV-1 RNA.

234 At the clinical threshold of 1000 copies/mL, sensibility of FTA cards in this study was
235 seen at 78.6% with Roche, which was slightly less than that observed with Abbott
236 assay (Se = 92.3%) and in already most available literature using Whatman 903[®]
237 cards [15,21–24,28]. Inversely, our results were higher than that observed in Vietnam
238 using Whatman 903[®] cards with Roche assay [29]. Additionally, the contribution of
239 HIV cell-associated DNA and RNA could be the reason for slightly lower sensibility
240 for Roche assay. Another possible explanation of the sensitivity observed with Roche
241 could be the elution protocol used in the current study. Following manufacturers'
242 instructions, time to incubation of DBS is 10 minutes in thermomixer at 1000 rpm,
243 56°C.

244 Both Roche and Abbott assays in this study showed a good correlation and
245 agreement between FTA cards and plasma values which is similar to other studies

246 comparing DBS (using Whatman 903[®] cards) to plasma specimens with Roche and
247 Abbott assays [12,14,15,20].

248 Our study has some limitations. First, the sample size was restricted. Second, FTA
249 cards were not blotted via finger-prick blood. Third, our study was a laboratory-based
250 study, so the impact of FTA cards sample transport conditions was not evaluated.
251 This study gives preliminary elements to investigations for a longitudinally designed
252 study with a stronger power, incorporating additional factors, such as transport and
253 storage under local conditions, to further evaluate FTA cards specimens for HIV-1 VL
254 testing.

255

256 **Conclusion:**

257 In summary, we demonstrated the feasibility of using FTA cards for HIV-1 VL testing.
258 FTA cards was found to be a sensitive and specific alternative to plasma testing for
259 HIV-1 VL testing using Abbott assay. Both Roche and Abbott assays showed a good
260 correlation and agreement between FTA cards and plasma values. This information
261 is relevant when considering how to improve access to VL testing by diversifying the
262 type of filter papers available in resource-limited settings.

263 A study which will increase the testing population size and compare the use of
264 Whatman FTA[®] to Whatman 903[®] cards specimens for VL testing using both Roche
265 and Abbott assays is planned for the future.

266

267 **Acknowledgments**

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271 conclusions in this paper are those of the authors and do not necessarily represent
272 the views of the PSSLS-IST.

273

274 **Conflict of interest**

275 The authors declare that they have no competing interest.

276

277 **Authors Contributions**

278 L.S. conceived and designed the experiments; A.Y. actively participated to the
279 specimen collection and the study design; A.Y., M.C, G.K.D., H.S., D.C., K.O.

280 performed the experiments. A.Y. analyzed the data; A.Y. and L.S. wrote the
281 manuscript. All authors read and approved the final manuscript.

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397 **Figures and tables legends**

398 **Figure 1:** Comparison between Whatman FTA[®] cards and plasma specimens in HIV-
399 1 RNA. The boxplot to the left (**A**): using Roche assay; the boxplot to the right (**B**):
400 using Abbott assay. The black points in the boxplot indicates the means values.

401 **Figure 2:** Correlation between FTA cards and plasma specimens in HIV-1 RNA
402 quantitation. **A**: using Roche assay; **B**: using Abbott assay.

403 **Figure 2:** Bland Altman analysis between FTA cards and plasma specimens in HIV-1
404 RNA quantitation. **A**: using Roche assay; **B**: using Abbott assay.

405 **Table 1:** HIV-1 viral load using Whatman FTA[®] cards and plasma specimens with
406 Roche assay

407 **Table 2:** HIV-1 viral load using Whatman FTA[®] cards and plasma specimens with
408 Abbott assay

409 **Table 3:** Sensitivity, specificity, positive predictive value, and negative predictive
410 value of Whatman FTA[®] cards compared with paired plasma specimen for HIV-1 viral
411 load testing at a 1000 copies/mL medical decision point

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413 **Table 1:** HIV-1 viral load using Whatman FTA[®] cards and plasma specimens with
414 Roche assay

FTA cards specimens using Roche	Plasma specimens using Roche			Total	p value
	Not detected	Moderate	High		
Not detected	39 (83.1)	7 (14.9)	1 (2.1)	47 (100.0)	< 2.2 10 ⁻¹⁶
Moderate	16 (53.3)	9 (30.0)	5 (16.7)	30 (100.0)	
High	0	0	22 (28.3)	22 (100.0)	

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417 **Table 2:** HIV-1 viral load using Whatman FTA[®] cards and plasma specimens with
418 Abbott assay

FTA cards specimens using Abbott	Plasma specimens using Abbott			Total	p value
	Not detected	Moderate	High		
Not detected	60 (83.3)	10 (13.9)	2 (2.8)	72 (100.0)	< 2.2 10 ⁻¹⁶
High	1 (3.7)	2 (7.4)	24 (88.9)	27 (100.0)	

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421 **Table 3:** Sensitivity, specificity, positive predictive value, and negative predictive
422 value of Whatman FTA[®] cards compared with paired plasma specimen for HIV-1 viral
423 load testing at a 1000 copies/mL medical decision point

FTA cards (copies/mL)	Plasma (copies/mL)		Total	Se, Sp, PPV, and NPV
	≤ 1000	≥ 1000		
ROCHE	≤ 1000	22	0	Se = 78.6%; Sp = 100.0%, PPV = 100.0%; NPV = 92.2%
	≥ 1000	6	71	
	Total	28	71	
Abbott	≤ 1000	24	3	Se = 92.3%; Sp = 95.9%; PPV = 88.9%; NPV = 97.2%
	≥ 1000	2	70	
	Total	26	73	

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Figures

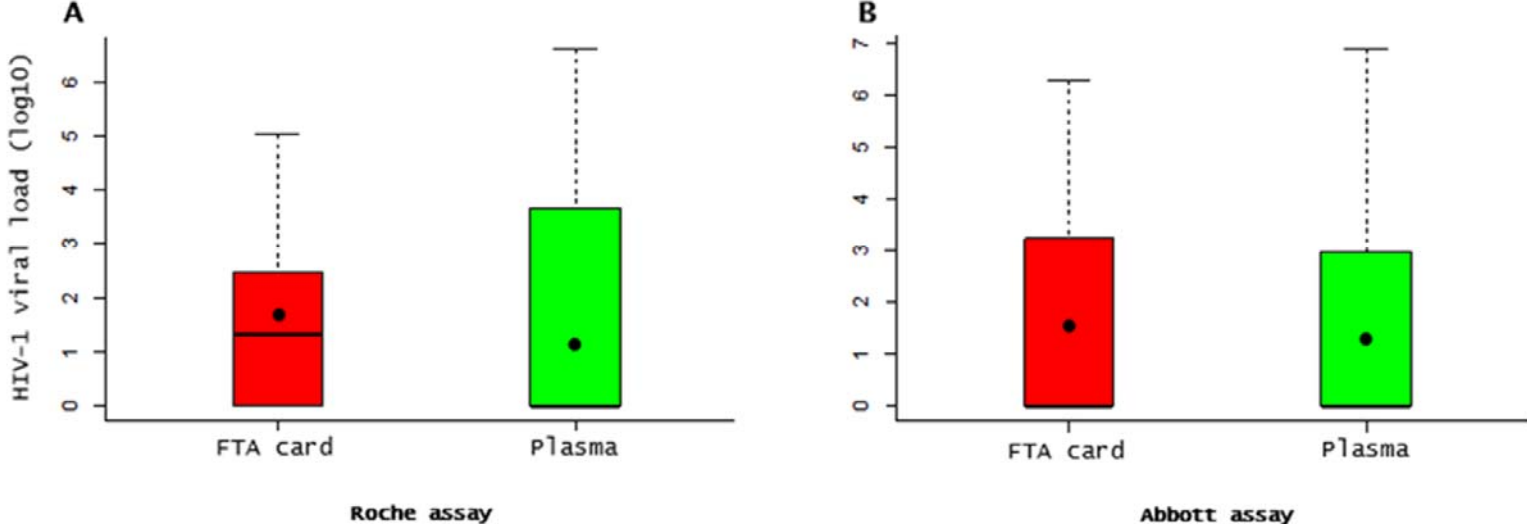


Figure 1: Comparison between Whatman FTA[®] cards and plasma specimens in HIV-1 RNA. The boxplot to the left (A): using Roche assay; the boxplot to the right (B): using Abbott assay. The black points in the boxplot indicates the means values.

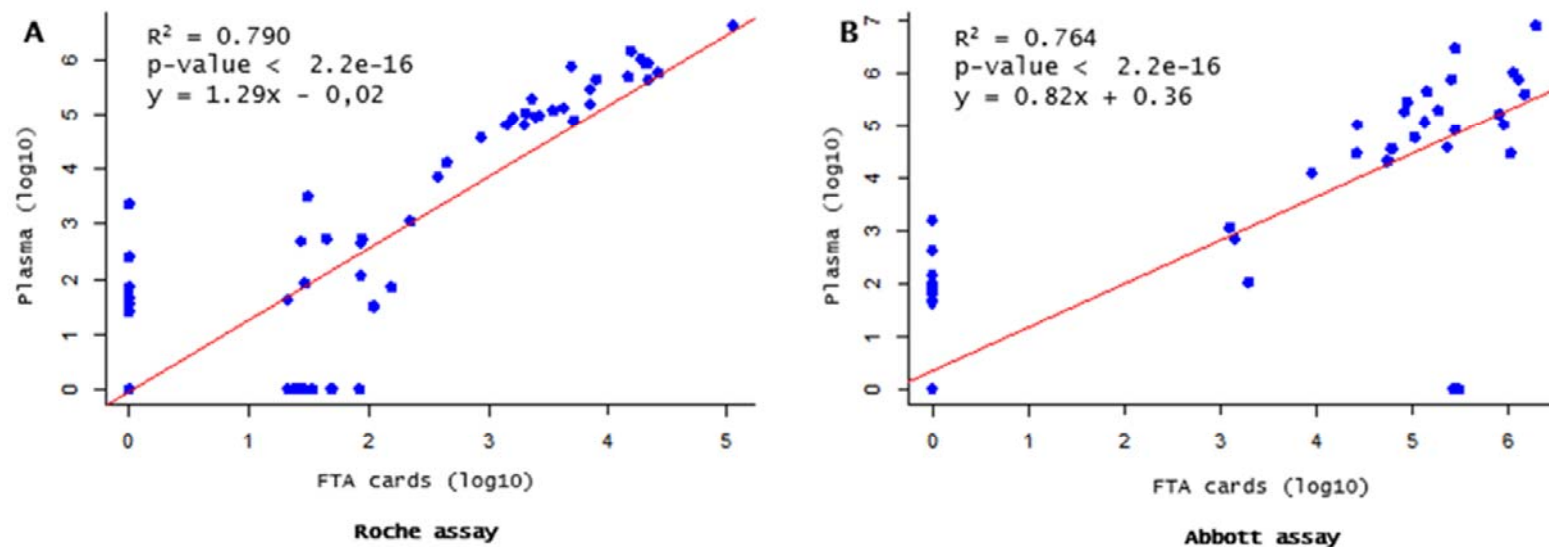


Figure 2: Correlation between FTA cards and plasma specimens in HIV-1 RNA quantitation. **A:** using Roche assay; **B:** using Abbott assay.

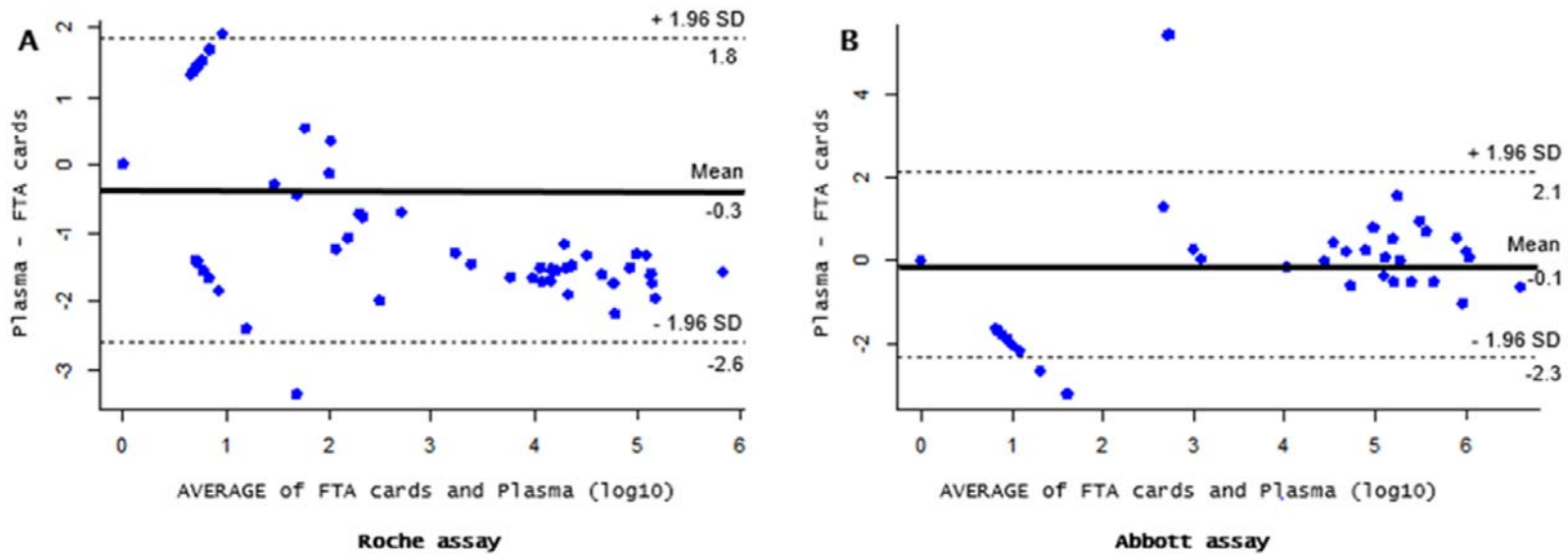


Figure 3: Bland Altman analysis between FTA cards and plasma specimens in HIV-1 RNA quantitation. **A:** using Roche assay; **B:** using Abbott assay.