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1 **Performance of the Xpert HBV Viral Load assay versus the**
2 **Aptima Quant assay for quantifying hepatitis B virus DNA**

3

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21 **ABSTRACT**

22 Quantification of HBV DNA is used for initiating and monitoring antiviral treatment. We have
23 evaluated the Xpert HBV Viral Load (VL) assay on the GeneXpert instrument.

24 We estimated its limit of detection to be 7.5 IU/ml Reproducibility was 1.1% - 12.7%, as
25 assessed by the coefficients of variation for three different samples. The assay was linear
26 from 2 to 8 log₁₀ IU/ml for HBV genotypes A to F. Its clinical performance was evaluated by
27 testing prospectively 100 HBV DNA-positive samples with the Xpert HBV VL and Aptima
28 Quant HBV assays. The results from the two assays were correlated, with a modest bias
29 (-0.10 log₁₀ IU/mL) between them by Bland-Altman analysis. Patient monitoring with 80
30 samples performed with both assays gave similar patient profiles with trends in the same
31 direction.

32 The Xpert HBV Viral load assay is reliable enough for quantifying HBV DNA in clinical practice.

33

34 **Key Words:** Hepatitis B virus, molecular diagnosis, GeneXpert instrument

35 **Abbreviations**

36 ALT: alanine amino transferase

37 EASL: European Association for Study of the Liver Diseases

38 HBV: Hepatitis B virus

39 POC: point-of-care

40 TMA: transcription mediated assay

41 VL: Viral load

42

43 **1. Introduction**

44 Hepatitis B virus (HBV) infections are a major cause of chronic liver disease that
45 affects almost 250 million individuals worldwide (1). Chronic HBV infection is associated with
46 a wide spectrum of liver diseases, from HBeAg-positive or negative chronic hepatitis B with
47 high HBV DNA concentration to a low-viremia HBe antigen negative chronic HBV infection,
48 which may evolve toward cirrhosis and hepatocellular carcinoma (2). HBV-related end-stage
49 liver disease and hepatocellular carcinoma are responsible for more than 750,000 deaths
50 worldwide each year. Hepatitis B viruses are presently divided phylogenetically into 9
51 genotypes, A-I , with a putative 10th genotype, "J", isolated from a single individual, based
52 on an intergroup divergence >7.5% across the complete genome (3).

53 International practice guidelines recommend using sensitive nucleic acid
54 amplification techniques for detecting and quantifying HBV DNA in clinical practice (4, 5).
55 Accurate assays for HBV DNA quantification are essential for the management of HBV
56 infections, establishing the prognosis for HBV-related liver disease, guiding decisions
57 concerning treatment, and monitoring the virological response to antiviral treatment (4, 5).
58 According to EASL guidelines, treatment requires HBV DNA >2,000 IU/ml, elevated ALT
59 and/or at least moderate histological lesions. However, patients with HBV DNA
60 >20,000 IU/ml and elevated ALT should start treatment regardless of the degree of fibrosis.
61 Several commercial nucleic acid amplification tests (NAATs) are currently available for
62 quantifying HBV DNA in patients' clinical samples (6-14). And, molecular techniques are
63 easier to implement compared to several years ago, reagents have to be reconstituted, like
64 for the Aptima Quant HBV DNA assay, and often require several controls per day. Less-
65 complex and inexpensive assays are needed for more widespread use of targeted, routine
66 HBV DNA monitoring and for point-of-care (POC) use (15, 16).

67 This study evaluated the analytical and clinical performance of the new Xpert HBV
68 Viral Load (VL) assay (Cepheid Inc. Sunnyvale, CA, USA) on the GeneXpert system (Cepheid).

69

70 **2. Patients and Methods**

71 **2.1 HBV assays**

72 *Xpert HBV DNA VL:* This assay is a semi-automated, real-time PCR test that quantifies HBV
73 DNA. This single-target assay has a reported limit of quantification of 10 IU/ml, with a
74 dynamic range of 1–9 log₁₀IU/mL. The tests can be run on any GeneXpert instrument and
75 requires 0.6 mL plasma or serum per test (no dead volume). The technician pipettes the
76 plasma/serum into the assay cartridge and locks it into the GeneXpert system module. The
77 GeneXpert System can be configured with 1, 2, 3, 4, 8, 12, or 16 modules. Each module
78 contains a single cartridge for HBV DNA extraction, amplification and quantification. The
79 Xpert assay contains 2 internal controls but no positive or negative control is provided.
80 Extracted DNA cannot be reused. A two modules-instrument was used for the present study.

81

82 *Aptima HBV Quant assay:* The Aptima assay (Hologic Inc. San Diego, CA, USA), a real-time
83 transcription mediated amplification (TMA) assay is a dual-target assay designed to run on
84 the fully automated, random access Panther platform (Hologic, Inc.), and was used as the
85 comparator. Its limit of quantification is 10 IU/mL with a dynamic range of 1–9 log₁₀IU/mL
86 using 0.5 mL plasma or serum per test, with an additional 0.2 mL for the dead volume.
87 Extracted DNA cannot be reused. The Aptima HBV DNA assay implemented on the Panther
88 platform requires 6 calibrators and controls per day that are stored at -20°C. All other
89 reagents are reconstituted.

90

91 **2.2 Analytical performance of the Xpert HBV DNA assay**

92 *Analytical sensitivity.* The analytical sensitivity of the assay was determined with 5 dilutions
93 (50 IU/ml - 2.5 IU/ml) of the 3rd WHO International Standard for Hepatitis B Virus (NIBCS
94 10/264) in HBV negative plasma. Ten replicates of each HBV DNA serial dilution were tested
95 the same day on a single instrument.

96 *Specificity.* The specificity of the Xpert HBV DNA assay was assessed by testing, on the same
97 day, 20 HBs-antigen negative and HBe-antibody negative, clinical specimens (Architect,
98 Abbott Diagnostics, Chicago, IL, USA) from HIV and HCV negative donors.

99 *Reproducibility.* Plasma from three HBV DNA positive patients (HIV and HCV negative),
100 previously quantified using the Aptima assay, were separately diluted in HBV negative
101 plasma to obtain 3 samples with high (5 log₁₀ IU/ml), medium (4 log₁₀ IU/ml) and low (2.5
102 log₁₀ IU/ml) viral loads. These 3 samples were tested 20 times over a period of 20 days.

103 *Linearity and influence of the HBV genotypes.* Two clinical samples of each HBV genotype (A,
104 B, C, D and E) were diluted in negative plasma to obtain 2 series (A/A*, B/B*, etc.) of five 10
105 fold-dilutions. The resulting five concentrations of each genotype covered the linear range of
106 the assay. Three replicates of each dilution were tested with the Xpert HBV assay the same
107 day. HBV genotypes were determined as previously described in (17).

108

109 **2.3 Clinical performance of the Xpert HBV DNA assay**

110 Plasma samples were obtained from HBV chronically infected (Hepatitis B surface (HBs)
111 antigen and anti-hepatitis B Core (HBe) antibodies positive) patients attending the Toulouse

112 University Hospital. A total of 100 samples were collected, centrifuged at 2000g for 10
113 minutes. The plasma was kept at 5°C for a maximum of 48h until tested with the Aptima
114 assay, and stored again at 5°C for a maximum of 24h until tested with the Xpert assay.

115 We also tested 80 stored frozen samples (maximum freezing time: 1 year at -80°C) that had
116 been collected in 19 HBV DNA positive patients being monitored during their treatment for
117 HBV infection and previously tested with the Aptima assay.

118 Biological material and clinical data were obtained only via standard viral diagnostics
119 following a physician's order (no supplemental or modified sampling). Data were analyzed
120 anonymously. The French Public Health law (CSP Art L 1121-1.1) does not require written
121 informed consent from the patients for such a protocol.

122

123 **2.4 Workflow Study**

124 The time-to-result (time period between arrival of the sample at the lab to the final report)
125 for the Xpert HBV and Aptima assays were assessed over one work week (Monday to Friday).

126 The samples were sent to the lab for registration, centrifugation, aliquoting, bar coding and
127 then analysis. Time-to-result was defined as the time period between arrival of the sample
128 at the reception to the final report with HBV DNA result on the laboratory information
129 system. The Xpert assay was performed with a 2-module GeneXpert instrument every day
130 from 8:00 to 16:00 hours. The Aptima assay was performed on Monday, Wednesday and
131 Friday on the Panther automate between 11:00 and 19:00 hours to minimize the number of
132 controls/calibrators and cost associated.

133

134 **2.5 Statistical analysis**

135 HBV DNA quantities were \log_{10} transformed prior to analysis. The limit of detection (LOD)
136 was determined by Probit analysis of the WHO standard HBV sample data. Linearity was
137 estimated by linear regression. The values obtained from the Xpert HBV VL and Aptima
138 assays were analyzed by Deming linear regression. A Bland-Altman analysis (scatter plot of
139 the differences between the paired measurements plotted against their means) of positive
140 samples obtained by the two methods was used to assess graphically the magnitude of
141 disagreement between them and estimate the overall bias.

142

143 **3. RESULTS**

144 **3.1 Analytical performance**

145 *Sensitivity.* The detection rates were 100% for 50 IU/ml, 20 IU/ml and 10 IU/ml replicates,
146 80% for 5 IU/ml replicates, and 60% for 2.5 IU/ml replicates (Table 1). Probit analysis of the
147 50 replicates of the diluted NIBSC standard gave an LOD of 7.5 [CI 95%: 4.7 -564.6] IU/ml for
148 the Xpert HBV assay.

149 *Specificity.* All the 20 negative samples tested negative for HBV DNA: specificity = 100%.

150 *Reproducibility.* Reproducibility (\log_{10} standard deviation) was determined by testing 20
151 replicates over 20 days. The log 10 standard deviation for 5.1, 3.9 and 2.2 \log_{10} IU/ml was
152 0.06, 0.10 and 0.28 \log_{10} IU/ml, respectively (Table 2) .

153

154 *Linear quantification and genotype inclusivity*

155 The linearity of the Xpert assay was evaluated with serial dilutions of 10 clinical samples of
156 genotypes A to E (Fig. 1), the most common genotypes in Europe [10]. Linear regression
157 analysis gave a coefficient of determination (R^2) of > 0.99 for all the samples (Figure 1).

158

159 **3.2 Clinical performance**

160 We evaluated the accuracy of the Xpert HBV by comparing the HBV DNA concentrations
161 obtained by the Xpert and Aptima assays; they were linearly related ($p < 0.0001$; Deming
162 regression equation: $Xpert = 1.063 \times Aptima - 0.103$) (Figure 2A). Bland-Altman analysis
163 (Figure 2B) indicated that the results for 95/100 samples gave results within the 95% limit of
164 agreement of the assays (-0.59 to 0.81 IU/mL), with a modest bias ($-0.10 \log_{10}$ IU/mL)
165 (Figure 2B). One out of 3 samples that were over-quantified with the Xpert assay belongs to
166 HBV genotype A. The 2 under-quantified samples with the Xpert assay belong to genotypes
167 D and E. The genotype was available for 4 additional samples that were within the 95% limit
168 of agreement of the assays. They belong to genotype A ($n=1$) or D ($n=3$) suggesting that
169 discrepant result was not linked to a particular genotype.

170

171

172 **3.3 Patient monitoring**

173 A total of 80 samples from 19 patients chronically infected with HBV were tested with the
174 Xpert HBV and Aptima HBV Quant assays. The HBV DNA dynamics of all the patients were
175 parallel (Fig 3). The resulting data, HBV DNA load (\log_{10} IU/mL) versus time (weeks), gave
176 similar profiles with trends in the same direction and overlapping values, except for patient
177 #4. The Xpert assay underestimated all the samples from this patient that was infected with
178 HBV genotype A (difference $> 1.5 \log_{10}$ IU/ml) (Fig 4). A slight underestimation with the Xpert
179 assay was observed for patients #1, #2, #3, #5, #6 and #12. The virus infecting patients 6 and
180 12 couldn't be genotyped. Patients #3 and #5 were infected with genotype A, patient #1 was
181 infected with genotype D and the patient #2 was infected with genotype E. The genotype
182 was available for 1 additional patients with concordant result. It belong to genotype A.

183

184 **3.4 Workflow study**

185 A total of 50 samples had volumes that were large enough for both assays and were tested.
186 The tests were carried out by 2 separate technicians at different times during a one-week
187 period. The Xpert assays were performed every day. The Aptima assays were performed on
188 Monday, Wednesday and Friday. Overall, the mean time-to result was 8h (range: 2-24h) for
189 the Xpert assay and it was 23h (range: 5-51h) for the Aptima assay. Thus, the Xpert assay
190 required an average of 15h less time-to result than did the Aptima assay. The mean time-to-
191 result for the Xpert assay and for the Aptima assays was 8h and 10h respectively for samples
192 arrived on Monday, Wednesday and Friday whereas it was 7.5h and 31h respectively for
193 samples arrived on Tuesday and Thursday.

194 **4. Discussion**

195 We found the analytical performance of the Xpert assay for detecting and quantifying
196 HBV DNA to be specific, reproducible and linear with a low limit of detection. We also found
197 a strong, significant correlation between the HBV DNA concentrations obtained with the
198 Xpert and Aptima Quant assays.

199

200 The analytical sensitivity of the Xpert assay, assessed by testing plasma dilution was
201 excellent, with a lower limit of detection (LOD) in keeping with the manufacturer's stated
202 value (10 IU/mL). This LOD is similar to that of the most commonly used HBV DNA platforms:
203 the Abbott RealTime HBV test, the Roche Cobas AmpliPrep/Cobas TaqMan HBV test, the
204 Roche Cobas TaqMan HBV test with the HighPure system, and the Qiagen Artus HBV (11)
205 and Aptima Quant HBV tests (8). According to the Xpert assay's package insert, the LOD is
206 3.2 IU/ml for the plasma EDTA and 5.99 IU/ml for the serum.

207 While we tested only a small number of negative samples to assess specificity, we
208 found no false positive results. A previous report found false-positive results with all the
209 assays evaluated (11) but they were more frequent for assays that used manual extraction
210 methods (4/28 for the HighPure TaqMan test and 5/28 for the Artus HBV assay, and only
211 1/28 for the Real-Time and 2/28 for the AmpliPrep TaqMan assays). The simplicity and the
212 unitary format of the of the Xpert HBV assay should theoretically limit the risk of sample
213 cross-contamination as all the steps (extraction and PCR) take place within the same
214 cartridge in an independent module on the GeneXpert instrument.

215 Assay reproducibility was good, particularly for high-load controls; the coefficient of
216 variation was <3% for controls with HBV DNA $\geq 3 \log_{10}$ IU/mL. The Aptima and the COBAS
217 Ampliprep/COBAS TaqMan assays have given higher CVs (6). The Xpert assay was also linear

218 over a wide range of concentrations (2–8 log₁₀IU/mL) across all the genotypes tested (A to
219 E).

220 The clinical performances of the Xpert and Aptima assays were very well correlated,
221 with most (95%) of the samples falling within the 95% limit of agreement of the assays in the
222 Bland-Altman analysis. The average difference between the assays was very small (-0.10
223 log₁₀IU/mL). A similar difference was reported between the Aptima and COBAS
224 Ampliprep/COBAS TaqMan assays: 0.09 to 0.19 log₁₀IU/mL (6, 8, 14). Monitoring treatment
225 is an important part of chronic HBV patient management as it is used to determine the
226 efficacy of treatment and the occurrence of virological breakthrough (4, 5). We found that
227 the patient monitoring plots of HBV DNA produced by the Xpert HBV and Aptima assays
228 were similar for the majority of patients. The discrepant results (in the correlation and in the
229 monitoring studies) didn't seem to be related to a particular HBV genotype.

230 Real-time PCR or TMA assays have become the reference for quantifying virus
231 genomes in clinical practice according to international guidelines. However, commercially
232 available assays, although fully automated, use several separate analytical steps and require
233 at least one full day of work to complete the analysis, as shown in our workflow study with
234 the Aptima assay. Our workflow study indicated that the Xpert assay required a mean time to
235 results that was 15h shorter than with the Aptima on the Panther platform. The automated
236 real-time quantitative RT-PCR Xpert HBV assay could also be used as a point-of-care (POC)
237 test for patient management (outside a central laboratory, near the point of patient care).
238 The relatively uncomplicated nature of the Xpert HBV assay, coupled with its ability to
239 deliver same-day results, make it particularly well suited for use in low-income areas, and
240 also to avoid batch processing in industrialized countries. Were this assay to be deployed in
241 resource-constrained settings where GeneXpert platforms are already being used for

242 diagnosing HIV-1 or tuberculosis diagnostics, has the potential to increase access to HBV
243 testing worldwide. The diagnosis of HBV infection in these countries rely on serological
244 markers, the WHO guidelines in 2015 stated that a quantitative HBV DNA assay may help
245 treatment decisions. (18).

246 Our study presents some limitations. First, we did not have enough samples to retest
247 discrepant results. Secondly, the workflow study was a single center study and the number
248 of samples was low.

249 In conclusion, the Xpert HBV assay performed well and was quite comparable with
250 current reference tests. The minimal training and infrastructure required make it attractive
251 for use in resource-constrained settings. The quantitative Xpert assay is a promising tool for
252 monitoring the treatment of HBV patients; it could change how HBV DNA are monitored.

253

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255 **Declarations of interest.** None

256

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308

309

310 Table 1: determination of the limit of detection

<i>Dilution</i> <i>(UI/ml)</i>	<i>Number of replicates</i>	<i>Number of detected samples</i> <i>(%)</i>
50	10	10 (100%)
20	10	10 (100%)
10	10	10 (100%)
5	10	8 (80%)
2.5	10	6 (60%)

311

312

313

314 Table 2: Reproducibility

<i>Sample</i>	<i>Mean HBV DNA</i> <i>Log₁₀ IU/ml</i>	<i>SD</i> <i>Log₁₀</i> <i>IU/ml</i>	<i>CV (%)</i>	<i>Median</i> <i>Log₁₀ IU/ml</i>	<i>Range</i> <i>Log₁₀ IU/ml</i>
A	5.1	0.06	1.1	5.01	5.40-4.80
B	3.9	0.10	2.6	3.65	4.05 – 3.51
C	2.2	0.28	12.7	2.16	1.77- 2.86

315

316 Note: SD, standard deviation; CV, coefficient of variation.

317 **Figures legends:**

318 Figure 1. Xpert assay linearity using samples of different HBV genotypes (3 replicates per
319 samples).

320 Figure 2. Correlation between Xpert and Aptima quantitative results. All values are in log
321 IU/mL. (A) Deming regression analysis. The dotted line represents theoretical parity of
322 results. (B) Bland-Altman analysis. Dotted line represents the overall bias (average difference
323 between Xpert and Aptima results). The horizontal solid lines represent the 95% limit of
324 agreement.

325

326 Figure 3. Patient monitoring: virus loads of 19 treated HBV-infected patients: Xpert results
327 (solid line) and Aptima results (dotted line).

328

Figure 1

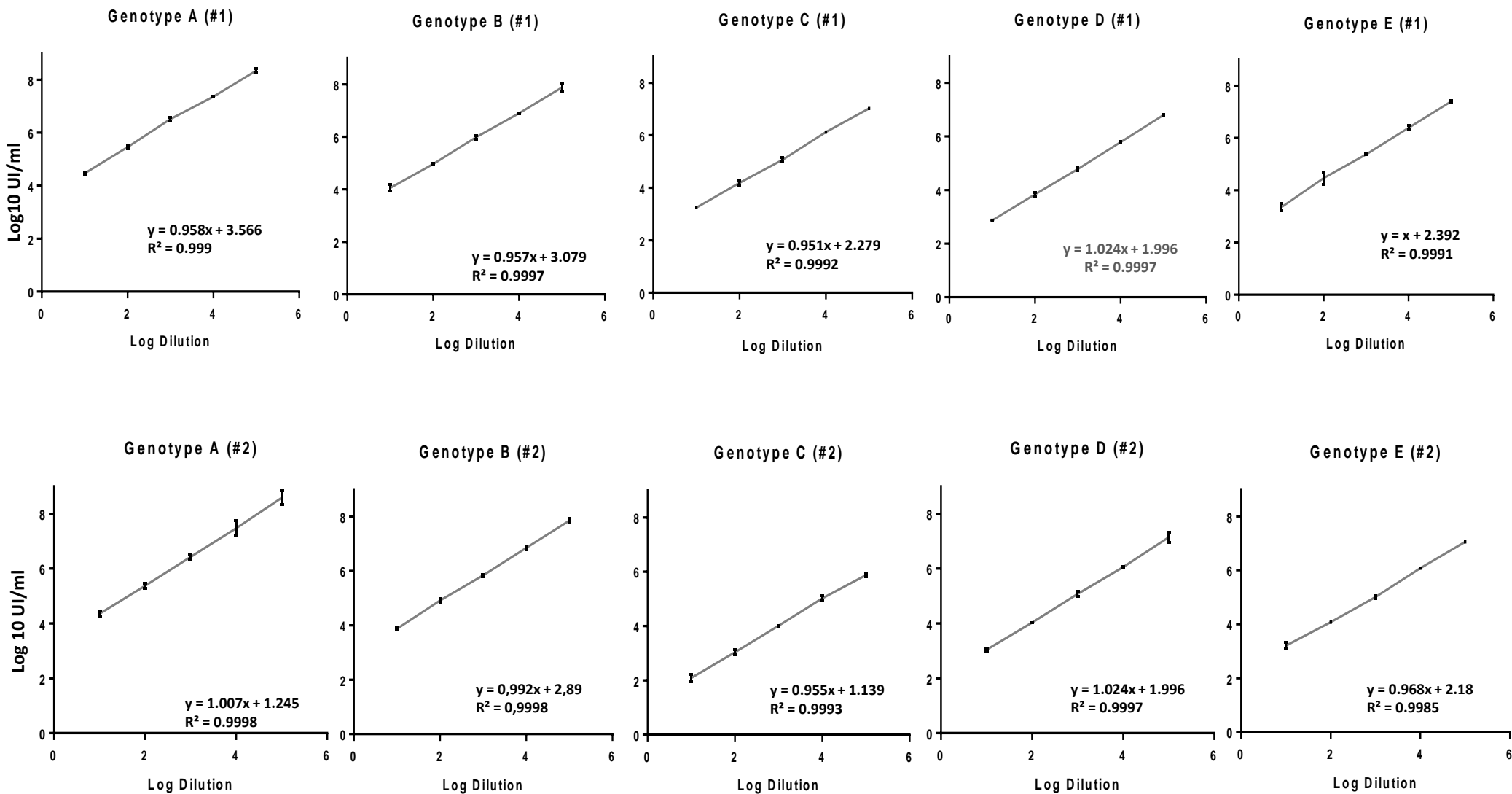
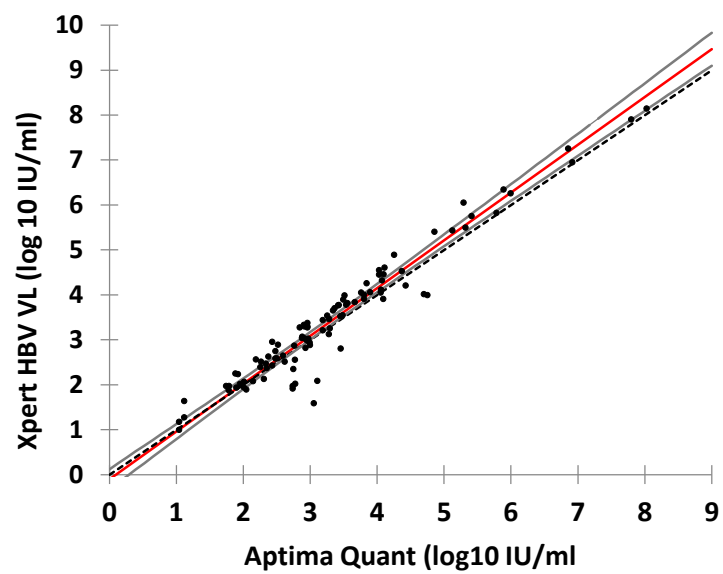


Figure 2

A/



B/

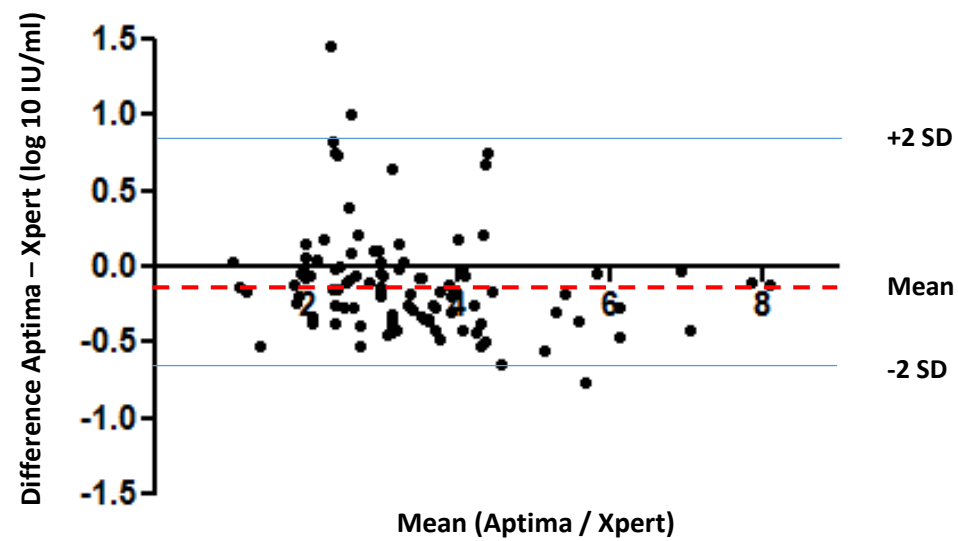


Figure 3

