Performance of the Novel Qiagen *artus* QS-RGQ Viral Load Assays Compared to That of the Abbott RealTi*me* System with Genetically Diversified HIV and Hepatitis C Virus Plasma Specimens

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We compared two novel Qiagen QS-RGQ viral load assays with the established Abbott RealTi*me* assays on a highly diversified panel of 121 human immunodeficiency virus (HIV) and 107 hepatitis C virus (HCV) specimens. The quantifications correlated well for all HIV and HCV types, but Qiagen yielded higher HCV concentrations than Abbott, predominantly in genotypes 4 and 5.

Quantification of human immunodeficiency virus (HIV) and hepatitis C virus (HCV) is a major tool for diagnostics, monitoring of antiviral therapy, and evaluation of transmission risks. Quantification of HIV is done routinely up to four times per year per patient. Quantification of HCV is done up to six times per year in patients undergoing antiviral treatment (17–19). Variation between the results determined by different assays bears the risk of misguiding treatment decisions (19). Reagent costs for all viral load assays are high (in the range of $50 to $100 per test), highlighting the need for robust systems that avoid the necessity to retest patients (9, 10, 22). However, even widely used proprietary systems have shown shortcomings in their subtype/genotype coverage. One example was the prohibition of a novel real-time reverse transcription-PCR (RT-PCR) test for testing blood products in Germany in 2004 soon after its introduction and the ongoing difficulties represented by a revised version of this assay, including a 2011 recall (1, 5).

A more recent entry in this field is Qiagen, offering real-time RT-PCR-based assays for HIV and HCV. In contrast, the Abbott RealTi*me* assays were introduced into the market in 2005 and have become known for their good performance on diverse HIV subtypes and HCV genotypes (16, 20). In this study, we have compared the quantitative correlations of the Abbott RealTi*me* (Darmstadt, Germany) and Qiagen *artus* QS-RGQ (Hilden, Germany) viral load assays on a genetically diversified panel of clinical HIV and HCV specimens.

Automated nucleic acid purification was done using the Qiagen QIAsymphony (DSP virus/pathogen midikit) and Abbott *m*2000sp platforms with a plasma input volume of 1.0 ml for Qiagen and 0.6 ml (HIV)/0.5 ml (HCV) for Abbott. The Qiagen real-time RT-PCR assays were the *artus* HIV-1 QS-RGQ, licensed for group M (subtypes A to H), and the *artus* HCV QS-RGQ. Both assays were run on a Rotor-Gene Q thermocycler. The values for the 95% lower limit of detection (LOD) given by the manufacturer were 76.4 international units (IU)/ml for HIV-1 and 36.2 IU/ml for HCV. The linear ranges of quantification were specified to extend from 250 to 10^8 IU/ml for HIV and 67.6 to 1.8×10^7 IU/ml for HCV. The Abbott assays were the RealTi*me* HIV, licensed for groups M (subtypes A to H), N, and O, and the RealTi*me* HCV, both run on an *m*2000*rt* thermocycler. The values for the 95% lower limit of detection (LOD) indicated by the manufacturer were 69.3 IU/ml for HIV and 12.0 for HCV. The linear ranges of quantification were given as 69.3 to 1.7×10^7 IU/ml for HIV and 12.0 to 10^8 IU/ml for HCV.

TABLE 1 Sample characteristics and viral load deviation per HIV/HCV type

Sample category	No. of reference panel specimens (NIBSC/Essen)[a]	No. of clinical plasma specimens	Log10 deviation, Qiagen vs Abbott (median/IQR)[b]	Significance[d]
HIV subtype/circulating recombinant form (CRF)/group				
A	1	14	−0.11/0.85	0.307
AB		8	0.24/0.32	0.017*
AE	1	13	0.26/0.53	0.064
AG		17	0.37/0.32	0.002**
AG/GH	1		0.17	
B	1	17	0.07/0.33	0.306
BF		1	0.35	
C	1	10	0.00/0.37	0.286
CD		3	0.48	0.109
CRF06 (A/G/J/K)		1	0.37	
CRF09 (A/G/U)		2	−0.47	0.180
CRF11 (A/CRF01/G/J)		1	0.13	
D	1	17	0.18/0.70	0.306
DF		3	0.20	0.109
F	1	1	0.17	0.655
G	1	3	0.47/0.46	0.144
N	1		−0.29	
O	1		−3.74[c]	
Total	10	111		
HCV Genotype				
1	3	17	0.49/0.17	<0.001***
2	5	18	0.27/0.18	<0.001***
3	2	22	0.29/0.34	0.001**
4	2	20	0.55/0.28	<0.001***
5	2	10	0.71/0.35	0.002**
6	2	4	0.25/0.64	0.345
Total	16	91		

[a] NIBSC (National Institute for Biological Standards and Control, Hertfordshire, United Kingdom), $n = 10$ for HIV and $n = 6$ for HCV (one each of genotypes 1 to 6); Essen (University of Essen, Germany), $n = 10$ for HCV (genotypes 1 [$n = 2$] and 2 [$n = 4$] and one each of genotypes 3 to 6).
[b] Log10 Qiagen − Log10 Abbott. IQR, interquartile range (not given when specimen number below 4).
[c] Not quantifiable by Qiagen.
[d] The Wilcoxon signed rank test was generally applied due to the small numbers of specimens for some subtypes/genotypes. A significance level of <0.05 was assigned one asterisk, <0.005 two asterisks, and <0.001 three asterisks.
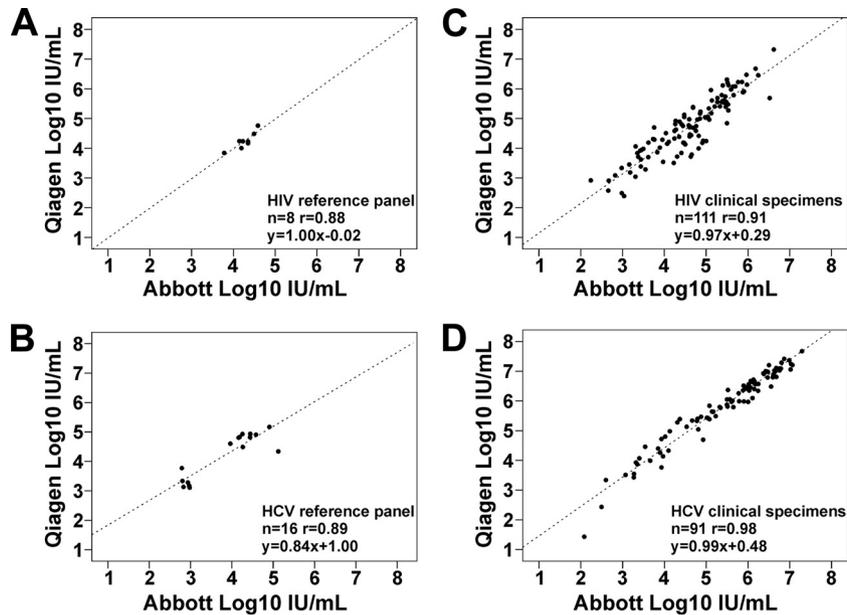
FIG 1 Correlation of viral loads as determined by Abbott RealTime and Qiagen *artus* QS-RGQ assays for HIV and HCV. Panels A and B show HIV and HCV reference panel specimens and panels C and D HIV and HCV clinical plasma specimens, respectively. In panel A, non-group M reference panel specimens were excluded because the Qiagen assay is not licensed for those. In panel C, three HIV specimens (one HIV CRF AG specimen quantifiable only by Abbott at 5.07 Log10 IU/ml and two subtype B specimens quantifiable only by Qiagen at 3.09 and 3.80 Log10 IU/ml, respectively) that were positive in one assay but repeatedly negative in the other assay were excluded. Specimen type, the number of samples (n), and Pearson's bivariate correlation coefficient (r) are given in the bottom right corner of each panel.

As shown in Table 1, the sample set comprised three genotype reference panels (total, $n = 16$ for HCV and $n = 10$ for HIV). For HIV, 111 clinical plasma specimens representing 15 different subtypes/circulating recombinant forms (CRF) were available. For HCV, 91 samples of all six genotypes were collected from routine diagnostics in 2002 through 2011 and a previous study (9). Clinical specimens were genotyped by sequencing of an approximate 1,600-bp fragment in the HIV *pol* gene (PCR primers available upon request) and a 389-bp fragment in the HCV NS5b gene (14), followed by comparison with online databases (2, 3). For one of the HCV reference panels (4), viral loads determined with Bayer Versant bDNA version 3.0 (bDNA) (6) and Roche Cobas Amplicor HCV Monitor version 2.0 (Amplicor) (13) were provided. HCV X-tail-based real-time RT-PCR (X-tail) viral loads were determined as described previously (9).

As a first step, correlations of Qiagen and Abbott viral loads determined on HIV and HCV reference panel specimens were analyzed. As shown in Fig. 1A and B, correlations between the two assays were highly statistically significant (two-tailed tests; $P < 0.01$ for both). Viral load correlations were equally statistically significant for clinical plasma specimens (Fig. 1C and D). However, one HIV CRF AG specimen was quantifiable by the Abbott test at 5.07 Log10 IU/ml but was repeatedly undetectable by Qiagen. Conversely, two subtype B specimens were quantified by Qiagen at 3.09 and 3.80 Log10 IU/ml but tested negative by Abbott.

Quantitative deviations by viral type were analyzed next. As shown in Fig. 2A and Table 1, all median deviations across different HIV types were within 0.5 Log10, a threshold considered acceptable in interassay comparisons. No significant ($P = 0.13$) differences of resulting mean viral loads, in a combined analysis including all HIV specimens, were identified by a paired t test, although some individual subtypes showed signif-

icant deviations (Table 1). Although the Qiagen assay has not been licensed for HIV groups N and O, a group N reference panel specimen was quantified at similar viral loads by Qiagen and Abbott. For HCV, the Qiagen assay showed higher quantification results across all genotypes relative to Abbott (Fig. 2B). In particular, HCV genotype 4 and 5 samples deviated by more than 0.5 Log10 (Table 1). The observed overall difference of means was statistically highly significant (paired t test; $P < 0.001$). To analyze whether these deviations occurred specifically between Qiagen and Abbott assays, viral loads of 10 HCV genotype reference panel specimens were compared by the inclusion of three additional assays. As shown in Fig. 2C, over-quantification of viral loads by Qiagen *artus* HCV QS-RGQ also occurred relative to the Bayer bDNA assay, the Roche Amplicor assay, and a recently described real-time RT-PCR assay in the HCV X-tail region (9). Nonetheless, quantitative deviations were also seen in comparisons between other assays, such as an underquantification of Abbott viral loads compared to the Roche Amplicor and in-house X-tail assays or variations of X-tail viral loads compared to Bayer bDNA and Amplicor.

This quantitative study compared the novel Qiagen *artus* HIV and HCV viral load assays with the Abbott assays as a gold standard. Quantitative performance of both reference panel and clinical plasma specimens was good for all HIV subtypes/CRF. For HCV, the overall Qiagen performance was good, but the assay systematically yielded higher concentrations in comparison to Abbott RealTime, mainly for genotypes 4 and 5. However, comparable or higher systematic deviations for non-1 HCV genotypes and non-B HIV subtypes between other well-established viral load assays are well known (7, 11, 15, 20, 21). Nevertheless, the relative overquantification of HCV needs to be communicated to health care professionals in order to prevent inadequate therapeutic de-
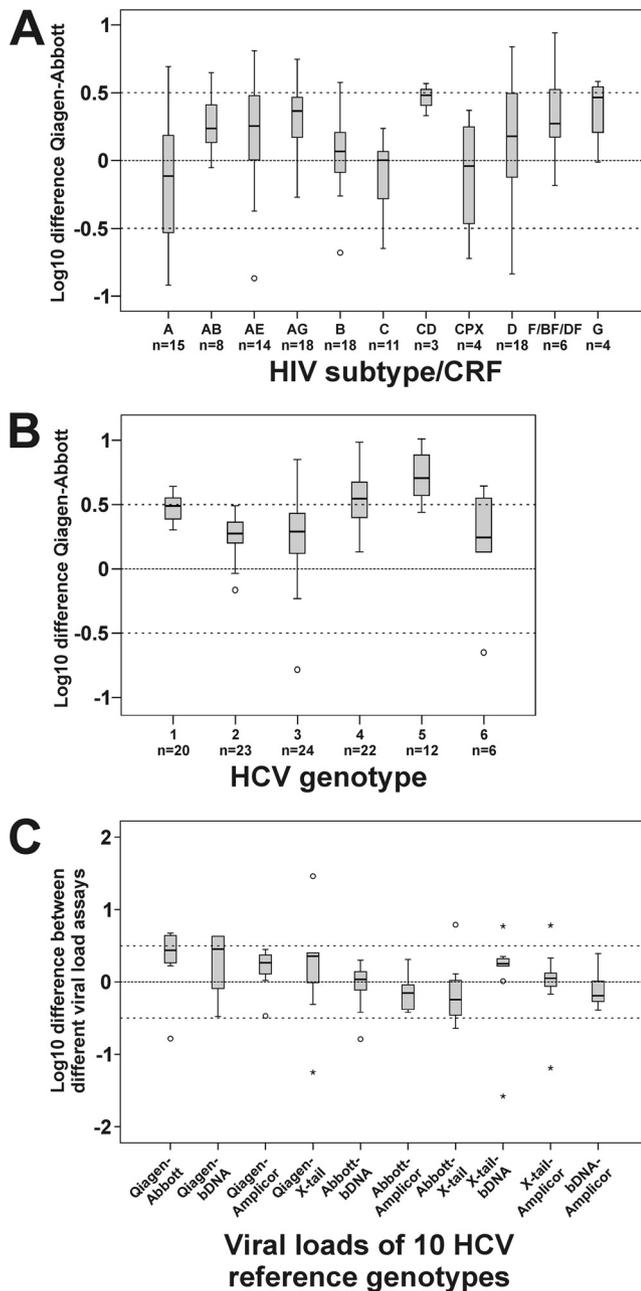
FIG 2 Quantitative deviations between Abbott RealTi*me* and Qiagen *artus* QS-RGQ viral load assays for HIV and HCV. Each box shows the median, interquartile range (box length, containing 50% of data), and whiskers, representing datum points within 1.5-fold of the interquartile range. Datum points beyond the whisker range are considered outliers, and extreme values (>3-fold beyond the interquartile range) are marked as circles and asterisks, respectively. Dashed lines on the *y* axis indicate 0.5 Log10 deviations from zero. (A and B) HIV subtypes/circulating recombinant forms (CRF) (A) and HCV genotypes (B) are indicated below the *x* axis with the numbers of samples tested per type (combined numbers from reference panel members and clinical specimens). For graphical reasons, HIV subtype F and circulating recombinant forms (CRF) containing F at any genomic position (one F, one BF, and three DF specimens) were summarized as F/BF/DF. The same was done for multiple recombinant CRF (one CRF06, two CRF09, and one CRF11 specimen), termed CPX. (C) Deviations between viral load determinations for *n* = 10 HCV genotype reference panel members (genotypes 1a, 1b, 2a, 2b, 2c, 2i, 3a, 4, 5a, and 6; University of Essen, Germany) by the Qiagen and Abbott assays and an additional three viral load assays are shown. bDNA, Bayer Versant bDNA version 3.0; Amplicor, Roche COBAS Amplicor version 2.0; X-tail, X-tail real-time RT-PCR.

cisions. An example may be the prolonged duration of antiviral treatment (12 instead of 6 months) routinely applied in cases of HCV genotype 1-infected patients presenting high pretreatment viral loads. Future assay versions might therefore benefit from a correction. The reasons underlying assay divergence remained unclear. While nucleotide mismatches at the oligonucleotide binding sites could explain this variation to some extent (8), this could not be assessed in this study, because neither Qiagen nor Abbott has released this proprietary information. An advantage of the Abbott assays remains the coverage of HIV groups N and O, although the Qiagen assay adequately detected one group N specimen. Furthermore, coverage of non-group M types may not be reliable for the Abbott assay (12).

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