



## Comparison of the QIAGEN *artus* HIV-1 QS-RGQ test with the Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 test v2.0

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### ABSTRACT

**Background:** Regular HIV-1 viral load monitoring is standard of care in the developed world for patients infected with HIV-1.

**Objectives:** Here we report a comparative evaluation of the established Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 v2.0 test (Roche Diagnostics Ltd, Burgess Hill, UK) and the new *artus* HIV-1 QS-RGQ test (QIAGEN Ltd, Crawley, UK).

**Study design:** 169 clinical EDTA-plasma samples were tested, all of known HIV-1 subtype.

**Results:** The mean overall  $\log_{10}$  c/ml difference was 0.10 (QIAGEN – Roche).

**Conclusion:** The *artus* HIV-1 QS-RGQ test compared well with the Roche TaqMan HIV-1 v2.0 test, and Bland–Altman analysis showed good agreement between the two systems across a wide range of subtypes.

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### 1. Background

HIV-1 viral load monitoring is performed with the principle aim of monitoring response to antiviral therapy where viral suppression below a certain cut-off, such as 50 copies/ml (c/ml), is desired.<sup>1–5</sup> Numerous publications have previously demonstrated the clinical performance of established automated HIV-1 viral load assays.<sup>6–9</sup>

The Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 test v2.0 (Roche HIV-1 v2.0 test) (Roche Diagnostics Ltd, Burgess Hill, UK) is a fully automated analyser for the quantification of HIV-1 groups M and O in human plasma. The AmpliPrep instrument is responsible for nucleic acid extraction and the TaqMan analyser is responsible for automated amplification and detection. Two regions of the HIV-1 genome are targeted with this assay (the *gag* gene and LTR region). The linear range of the assay is 20 to  $1 \times 10^7$  c/ml with a limit of detection (LOD) of 20 c/ml.

The QIAsymphony SP/AS system (QIAGEN Ltd, Crawley, UK) is a new docked system for the automated extraction of nucleic acid from clinical specimens and automated assay set-up. The SP module is responsible for nucleic acid extraction and the AS module integrates PCR set-up. HIV-1 viral load testing in human plasma (HIV-1 group M) can be performed in combination with the Rotor-Gene Q real-time PCR machine and the QIAGEN *artus* HIV-1 QS-RGQ kit (*artus* HIV-1 QS-RGQ test) which amplifies a 93 bp region of the

HIV-1 genome (LTR region). The linear range of this assay is 112.5 to  $4.5 \times 10^7$  c/ml with a LOD of 34 c/ml.

### 2. Objectives

We report a comparative evaluation of the Roche HIV-1 v2.0 test and the *artus* HIV-1 QS-RGQ test.

### 3. Study design

This evaluation was performed retrospectively on 169 HIV-1 RNA positive EDTA plasma specimens submitted to our laboratory for routine HIV-1 viral load testing. The samples were from patients with known HIV-1 subtypes, determined by routine genotypic resistance tests according to a validated in-house method analysing the protease and reverse transcriptase genome regions. Subtype prediction used the Stanford University HIV Drug Resistance Database HIVdb programme, REGA HIV-1 & 2 Automated Subtyping Tool and UCL STAR. EDTA blood tubes were centrifuged at 3000 rpm for 15 min prior to plasma being separated into 2 ml storage vials. Routine testing was performed by the Roche HIV-1 v2.0 test and the remaining plasma stored at  $-80^\circ\text{C}$ . Samples were retrieved from  $-80^\circ\text{C}$  storage, thawed, vortexed and pulse centrifuged before being processed on the QIAsymphony SP platform. 8 HIV-2 RNA positive EDTA plasma samples were tested retrospectively with the *artus* HIV-1 QS-RGQ test.

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### 3.1. Sample processing on the COBAS AmpliPrep/COBAS TaqMan docked 96 analyser

850  $\mu$ l of EDTA plasma were processed by this system (1050  $\mu$ l dead volume required). Nucleic acid was extracted by the COBAS AmpliPrep system prior to being automatically transferred to the COBAS TaqMan system for amplification and detection. All methods used were as stated in the COBAS AmpliPrep/COBAS TaqMan HIV-1 test v2.0 kit insert.<sup>10</sup> Results were given in c/ml and were transferred directly to the Laboratory Information Management System.

### 3.2. Sample processing on the QIASymphony SP/AS system

1000  $\mu$ l of plasma were processed by this system (1200  $\mu$ l dead volume required) prior to transfer to the AS module. All methods used were as stated in the *artus* HIV-1 QS-RGQ test handbook, which is available online.<sup>11</sup>

### 3.3. QIAGEN *artus* HIV-1 QS-RGQ test PCR set-up

PCR set-up was initiated, as stated in the *artus* HIV-1 QS-RGQ test handbook. Briefly 20  $\mu$ l of eluate/quantification standard/no template water control (NTC) were added to 30  $\mu$ l of mastermix. The tubes were sealed and transferred to the Rotor-Gene Q.

### 3.4. Rotor-Gene Q PCR

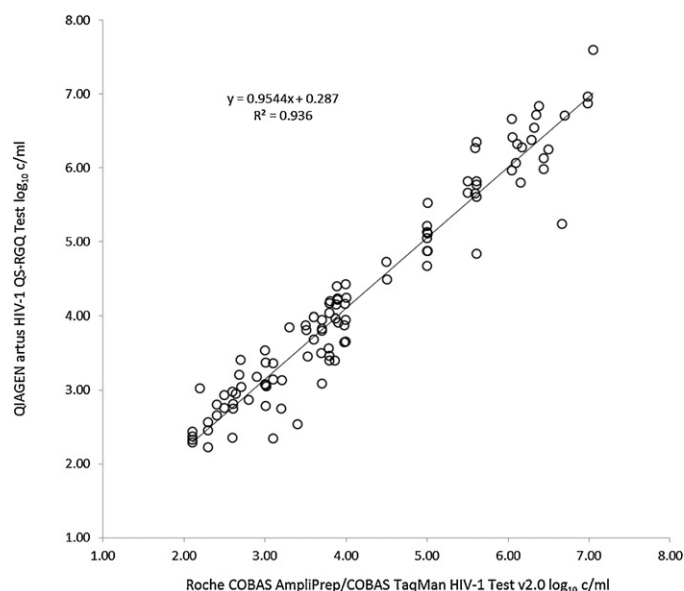
The *artus* HIV-1 QS-RGQ test was performed as stated in the *artus* HIV-1 QS-RGQ test handbook. Briefly a 50  $\mu$ l reaction volume was subjected to the following conditions: 1 hold step at 50 °C for 30 min, 1 hold step at 95 °C for 15 min, 50 cycles of 95 °C for 30 s, 50 °C for 60 s and 72 °C for 30 s. The data was analysed at a threshold of 0.04 for HIV-1 and 0.03 for the internal control. HIV-1 viral loads were calculated as IU/ $\mu$ l of eluate and converted to IU/ml of original sample. In order to compare results with those obtained with the Roche HIV-1 v2.0 test, a conversion factor of 0.45 copies/IU was used to convert the *artus* HIV-1 QS-RGQ test results to c/ml. The conversion factor is an approximation based on an average factor across the assay's dynamic range.<sup>11</sup>

### 3.5. Statistical analysis

Descriptive statistics were shown as mean values with associated SD values, or median values and ranges. Comparison between the  $\log_{10}$  c/ml viral load values obtained with both assays to show the strength of relation was shown in an XY scatter plot format. Bland–Altman analysis was used to show the order in which the data were in agreement (comparison of the differences between the 2 viral load results of the same sample versus the mean viral load of each sample).

## 4. Results

Results from the *artus* HIV-1 QS-RGQ test were compared with the Roche HIV-1 v2.0 test results. 169 HIV-1 RNA positive plasma samples were tested on both platforms. 66/169 (39%) samples had Roche HIV-1 v2.0 test results that were outside the *artus* linear range (all <112.5 c/ml). 103/169 (61%) samples had viral loads within the linear ranges of both tests. These 103 samples were from 99 patients. The mean linear range viral load of the *artus* HIV-1 QS-RGQ was 4.27  $\log_{10}$  c/ml compared with 4.17  $\log_{10}$  c/ml for the Roche HIV-1 v2.0 test.



**Fig. 1.** Correlation of the Roche HIV-1 test v2.0 and the *artus* HIV-1 QS-RGQ test (103 quantitative results).

#### 4.1. Linear correlation co-efficient

**Fig. 1** shows the values of samples with viral loads within the linear range of both assays in an XY scatter plot. Regression analysis gave a slope of 0.95 and showed that the y intercept was close to 0 (0.28). The correlation of results from both assays was good with an  $R^2$  value of 0.94.

#### 4.2. Bland Altman analysis

For all 103 EDTA plasma samples within the dynamic range of both assays, a plot of the differences between the values reported by both assays versus the average value for each plasma sample was established by Bland Altman analysis (**Fig. 2**).<sup>12</sup> The mean  $\log_{10}$  c/ml difference between the 2 assays (*artus* HIV-1 QS-RGQ test – Roche HIV-1 v2.0 test) was 0.10 with a SD of 0.36. The  $-2$  SD and  $+2$  SD values for the individual  $\log_{10}$  differences were  $-0.62$  and  $0.82$ , respectively. 99/103 (96%) linear range samples were within the  $-2$  and  $+2$  SD ranges. The graph indicates no significant difference between the two assays.

#### 4.3. Linear range mean $\log_{10}$ c/ml difference

The linear range data was further broken down into defined ranges (**Table 1**). The mean  $\log_{10}$  viral load difference between the two assays was similar across all three viral load ranges.

#### 4.4. HIV-1 subtype breakdown

**Table 2** shows the mean  $\log_{10}$  difference of each subtype tested within the linear range (*artus* HIV-1 QS-RGQ test – Roche HIV-1

**Table 1**  
Linear range mean  $\log_{10}$  differences.

Roche HIV-1 v2.0 viral load ranges (c/ml)	Number of samples	Mean $\log_{10}$ difference ( <i>artus</i> – Roche) ( $\log_{10}$ c/ml)
112.5–2000	34	0.20
2001–200,000	41	0.04
>200,000	28	0.05
Overall	103	0.10

## Bland and Altman Analysis

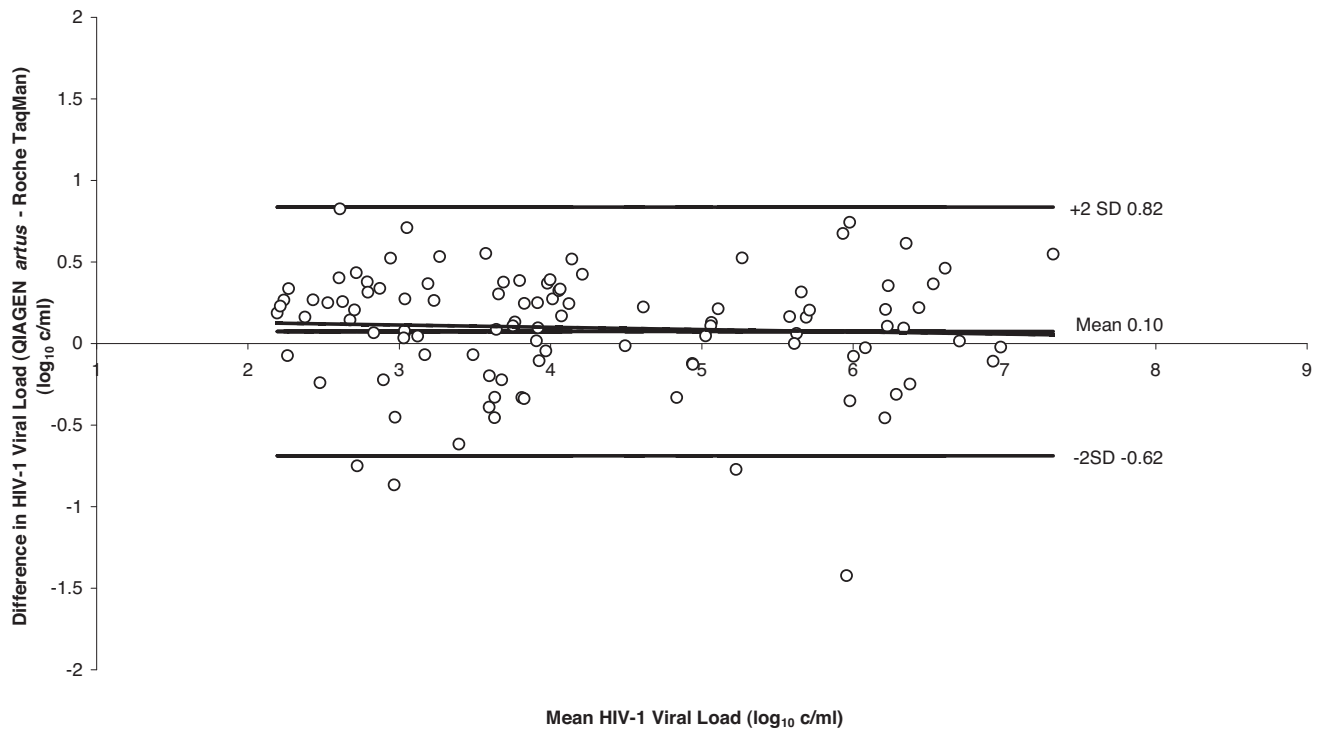


Fig. 2. Bland and Altman comparison of the *artus* HIV-1 QS-RGQ test with the Roche HIV-1 v2.0 test (103 quantitative results).

v2.0 test). 34% were subtype B (mean  $\log_{10}$  difference; 0.14), 19% subtype C (mean  $\log_{10}$  difference;  $-0.10$ ), 16% CRF02\_AG (mean  $\log_{10}$  difference; 0.35), 8% subtype A (mean  $\log_{10}$  difference;  $-0.24$ ) and 23% other subtypes (mean  $\log_{10}$  difference; 0.15).

#### 4.5. LLOQ results

66 EDTA plasma samples had Roche HIV-1 v2.0 results below the *artus* HIV-1 QS-RGQ test linear range ( $<112.5$  copies/ml). The subtypes in this group reflected the wide range described for the linear range samples. 4/66 samples were inhibitory when tested initially with the *artus* HIV-1 QS-RGQ test, with large ( $>1 \log_{10}$  c/ml) variation in HIV-1 viral load. Upon repeat testing this inhibition resolved and the HIV-1 viral loads were better aligned.

43 of the 66 samples had Roche HIV-1 v2.0 test results close to or below the LLOQ of the assay (20 c/ml). Specifically 16 samples were  $<20$  c/ml RNA detected and 27 samples were between 20 and  $<40$  c/ml. 17 of these 43 samples had an *artus* HIV-1 QS-RGQ test result of RNA not detected (8/17 were  $<20$  c/ml RNA detected by Roche HIV-1 v2.0 test). Of the remaining 26 samples, 19 were

$<34$  c/ml HIV-1 RNA detected and 7 were  $>34$  c/ml with a median HIV-1 viral load of 76 c/ml (range 37–217).

A further 20 samples with a Roche HIV-1 v2.0 viral load in the clinically relevant range of 50–112.5 c/ml (median Roche HIV-1 v2.0 result of 53 c/ml, range 50–101) were tested (Fig. 3) by the *artus* HIV-1 QS-RGQ assay. 6 of these samples were  $<34$  c/ml RNA detected and 16 samples were detected above the LOD with a median HIV-1 *artus* viral load of 71 c/ml (range 42–860).

#### 4.6. HIV-2 samples

8 EDTA plasma samples previously determined to be HIV-2 RNA positive by an in-house real time quantitative PCR were tested with the *artus* HIV-1 QS-RGQ test. The HIV-2 viral loads ranged from 1330 to 114,000 c/ml (Table 3). No amplification was observed with the *artus* HIV-1 QS-RGQ assay.

### 5. Discussion

Here we describe the results of a comparison of two commercially available automated analysers for the detection and

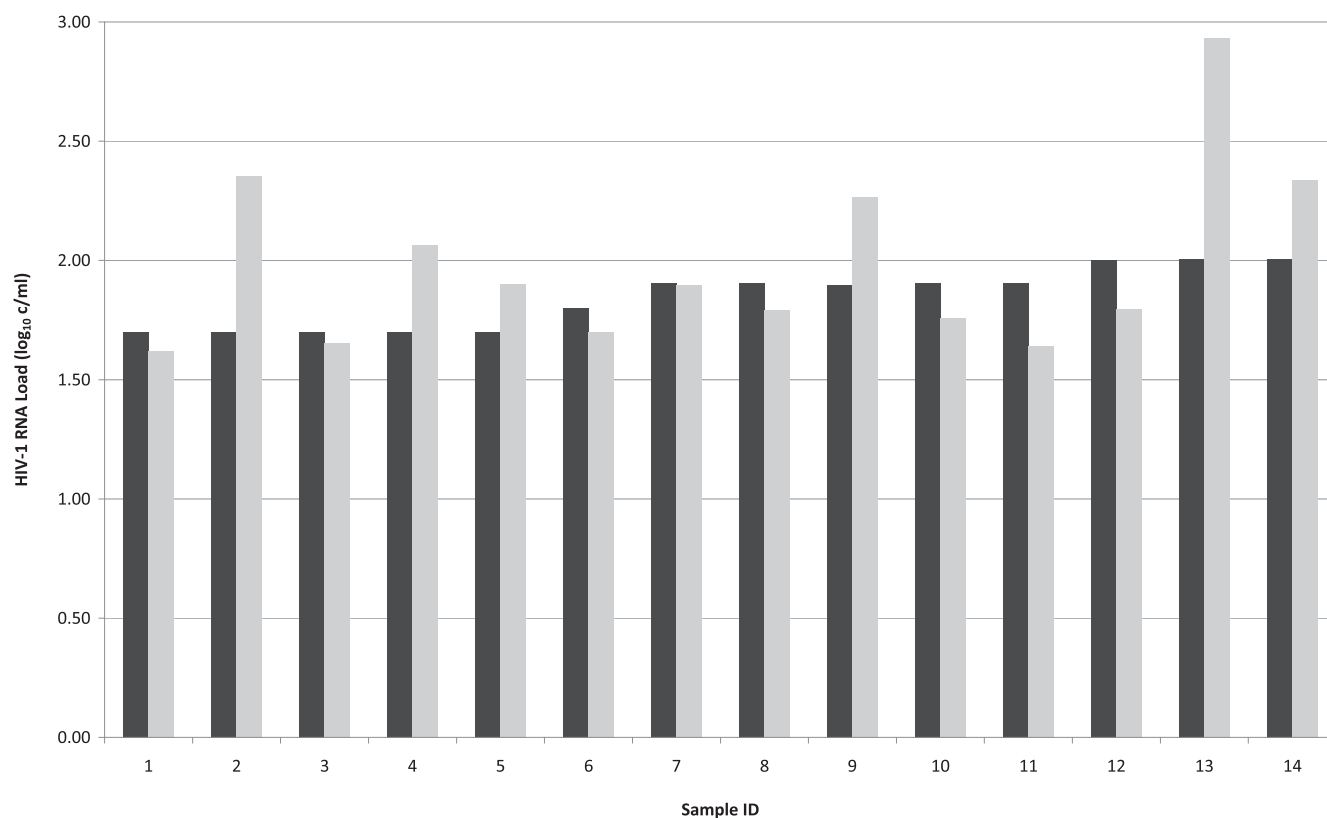
Table 2  
Mean log difference according to HIV-1 subtype.

HIV-1 subtype	Number of samples	Mean $\log_{10}$ difference ( <i>artus</i> – Roche) ( $\log_{10}$ c/ml)
B	35	0.14
C	20	$-0.10$
CRF02_AG	16	0.35
A	8	$-0.24$
Other <sup>a</sup>	24	0.15

<sup>a</sup> CRF01\_AE  $n=5$ , D  $n=5$ , A/CRF01\_AE  $n=2$ , A/D  $n=2$ , J/CRF01\_AE  $n=2$ , CRF20\_BG  $n=1$ , A/CRF16\_A2D  $n=1$ , G  $n=1$ , CRF01\_CPX  $n=1$ , F  $n=1$ , CRF06\_CPX  $n=1$ , CRF30\_0206  $n=1$ , G/CRF02\_AG  $n=1$ .

Table 3  
HIV-2 RNA positive samples.

Sample number	HIV-2 RNA viral load (c/ml)	<i>artus</i> HIV-1 QS-RGQ viral load (c/ml)
1	1770	RNA not detected
2	2140	RNA not detected
3	2080	RNA not detected
4	35,000	RNA not detected
5	114,000	RNA not detected
6	80,000	RNA not detected
7	1400	RNA not detected
8	1330	RNA not detected



**Fig. 3.** Comparison of 14 samples with Roche TaqMan HIV-1 test v2.0 viral loads between 50 and 112.5 copies/ml. Light grey; *artus* HIV-1 QS-RGQ test (log<sub>10</sub> c/ml). Dark grey; Roche HIV-1 v2.0 test (log<sub>10</sub> c/ml) (6 samples with *artus* HIV-1 QS-RGQ viral loads <34 c/ml not shown).

quantification of HIV-1 RNA. Both assays have the advantages of CE marking, automation, standardised reagents and having broad dynamic ranges over several log<sub>10</sub> values.

Comparing the results from the *artus* HIV-1 QS-RGQ test with the Roche HIV-1 v2.0 test in an XY scatter plot showed a good correlation between the two systems. Bland–Altman analysis also demonstrated that the difference between the two assays was low, and that overall the *artus* HIV-1 QS-RGQ test did not differ significantly from the Roche HIV-1 v2.0 test. These findings are similar to the study by Sandres-Sauné and colleagues, although we used the conversion factor of 0.45 copies/IU and the integrated AS module.<sup>13</sup>

Only 1/103 (0.97%) linear range samples gave a difference in HIV-1 viral load of more than 1 log<sub>10</sub> c/ml. This was a subtype C virus with a Roche HIV-1 v2.0 result of 6.67 log<sub>10</sub> c/ml and an *artus* HIV-1 QS-RGQ result of 5.25 log<sub>10</sub> c/ml. There was not enough sample volume available for re-testing on both systems, and this result would be unlikely to affect patient management. 4 samples exhibited inhibition with the *artus* HIV-1 QS-RGQ test and a wide discrepancy in viral load to the Roche HIV-1 v2.0 result. Upon repeat extraction and testing this inhibition resolved and the viral load results were more closely aligned, highlighting the importance of an internal control in qPCR to avoid the release of a false negative or under-quantified results.

17 samples in the study were *artus* HIV-1 RNA not detected, 9 of which had a Roche HIV-1 v2.0 result between 20 and 39 c/ml and 8 with a Roche result of <20 c/ml RNA detected. Given that 49 samples with a Roche HIV-1 v2.0 result <112.5 c/ml had corresponding detection of HIV-1 RNA by the *artus* test suggests that the latter still detects HIV-1 RNA below the LLOQ claims of the manufacturer and in line with the reported LOD of 34 c/ml. However, the validity of any viral load quantification with the *artus* test below its currently licensed LLOQ (112.5 c/ml) remains to be determined by the manufacturer. Indeed, plans to extend the linear range of the assay are

in development (personal communication, QIAGEN GmbH). Fig. 3 shows the viral loads obtained by both systems in the clinically relevant range from 50 copies/ml to the start of the *artus* HIV-1 QS-RGQ assay linear range at 112.5 copies/ml. These 20 samples had a median Roche HIV-1 v2.0 test result of 53 c/ml (range 50–101). 6 samples were detected below the 34 c/ml LOD of the *artus* HIV-1 QS-RGQ test and 14 were detected above this LOD with a median HIV-1 value of 71 c/ml (range 42–860).

8 HIV-2 RNA positive samples were processed with the *artus* HIV-1 QS-RGQ assay to verify specificity. No amplification was observed when these 8 samples of varying viral loads were tested, suggesting that there is no cross-amplification of HIV-2 RNA by this assay.

The mean log<sub>10</sub> c/ml difference was calculated both per viral load range and per subtype of HIV-1 tested. Both analyses showed no significant difference in mean viral loads associated with high or low HIV-1 titres or specific HIV-1 group M clades.

The new docked QIASymphony SP/AS platform increases the automation of QIAGEN *artus* real-time PCR assays, including the HIV-1 viral load test evaluated in this study. This potentially facilitates higher throughput as hands on time and operator dependant errors are reduced. An advantage of the QIASymphony SP/AS system is that as an open platform, in-house real time PCR assays can also be set up and a variety of different extraction protocols and sample types can be performed using the SP module.<sup>14</sup>

In conclusion HIV-1 RNA quantification by the QIAGEN *artus* HIV-1 QS-RGQ test correlated well with the Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 test v2.0.

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## Competing interests

D. Clark has received payment for lectures, travel and accommodation expenses from Roche Diagnostics Ltd and travel and accommodation expenses from QIAGEN Ltd. Although employed by Barts and The London NHS Trust at the time this evaluation, G Wall is now an employee of QIAGEN, GmbH.

## Ethical approval

Not required.

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