Comparison of the *artus* HIV-1 QS-RGQ and VERSANT HIV-1 RNA 1.0 assays for quantitative detection of human immunodeficiency virus type 1 in plasma samples

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---- SUMMARY ----

Several integrated diagnostic platforms to quantify human immunodeficiency virus type-1 viremia have been developed in recent years. We evaluated the performances of the *Artus* HIV-1 QS-RGQ assay, using the complete QIAsymphony RGQ workflow.

192 clinical plasma specimens and external control panel samples were analyzed, using the Artus assay and the routine Siemens VERSANT HIV-1 RNA 1.0 assay.

Three samples were excluded due to amplification inhibition. Among the remaining 189 specimens, 130 samples were detected as positive (above the limit of detection by both assays; median $\log_{10}$ difference: 0.01) and 18 samples were detected as negative. Eight samples (4.2%), all slightly above the limit of detection of the Versant assay, were negative with the Artus assay. The remaining 33 samples (beside 3 negative by Artus assay) were positive by both assays, but below the limit of detection at least in one of them. Results from the external panel samples showed a mean $\log_{10}$ variation of -0.18 and -0.45 for the Versant and the Artus assays, respectively.

As both assays showed highly correlated results, the QIAsymphony RGQ system, using the *Artus* HIV-1 QS-RGQ assay, could be considered a potential platform for HIV-1 RNA quantification in plasma.

KEY WORDS: Human immunodeficiency virus type-1 (HIV-1), *Artus* HIV-1 QS-RGQ assay, Quantitative real-time polymerase chain reaction (qPCR).

INTRODUCTION

Eight years after the first paper was published on the new Abbott quantitative real-time PCR (qPCR) assay for human immunodeficiency virus type 1 (HIV-1) RNA viral load determination in plasma, fluorescence-based qPCR technologies for the routine diagnosis of HIV-1 infection have become the most widely used methods (Garcia-Diaz, Clewley *et al.*, 2006; Pandori and Branson 2010; Sollis, Smit *et al.*, 2014). Moreover, novel diagnostic platforms have been developed to integrate sample handling within fully automated systems that minimize operator-dependent variability or, more importantly, the risk of sample contamination (Amendola, Marsella *et al.*, 2014). In addition, continuously improved nucleic acid extraction and amplification procedures have led to improved sensitivity and specificity, reducing the lower limit of detection (LOD) of the most recent assays to below 50 copies/ml, and improving the linearity of the assays to levels that until

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TABLE 1 - *Features of the molecular assays used in this study.*

	Siemens	Qiagen
Assay name	VERSANT HIV-1 RNA 1.0 (kPCR)	Artus HI Virus-1 QS-RGQ
Target sequence	Pol*	5'LTR
Sample volume	650 µL	1200 µL
Extraction volume	500 µL	1000 µL
Elution volume	70 µL	60 µL
Detection method	TaqMan	TaqMan
Standards	Two calibrators and Two external controls (high and low positives)	Four external quantification standards
Linear range	37 to 1.1×10^7 copies/mL	45 to 4.5×10^7 copies/mL
Analytical sensitivity	37 copies/mL	34.4 copies/mL

Long terminal repeat (LTR). *Integrase section of the *pol* gene.

very few years ago could not be measured. Importantly, quantification of HIV-1 viremia has become a mandatory need that impacts clinical management decisions and follow-up in patients with chronic HIV-1 infection undergoing antiretroviral therapy (ATR) (Thompson, Aberg *et al.,* 2010; Gianotti, Galli *et al.,* 2012; Gianotti, Galli *et al.,* 2013).

The aim of our study was to evaluate the clinical performance of the new quantitative *artus* HIV-1 QS-RGQ CE-IVD assay, using the complete QIAsymphony RGQ workflow (QIAGEN GmbH, Hilden, Germany) - Artus assay, for the detection and quantification of HIV-1 RNA in routine clinical plasma specimens. Results were compared with those obtained by the VERSANT HIV-1 RNA 1.0 (kPCR) assay (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA), - Versant assay, that is currently used as the routine assay in our laboratory. The basic features of the two molecular assays used in our study are summarized in Table 1.

MATERIAL AND METHODS

Specimen collection
Plasma samples were taken from adults for routine outpatient/inpatient monitoring or diagnostic workup, and subsequent monitoring for HIV-1RNA viral load in our Clinical Microbiology Laboratory. Plasma was collected in a 7-mL-EDTA BD Vacutainer tube, separated by centrifugation for 10 minutes at 3000 x g at room temperature within 6 hours of collection, and stored at +4°C for no longer than 48 hours

until the routine test was performed. Concurrently, a 1 µL aliquot of plasma was prepared and stored immediately at -80°C, for no more than 3 months, until retested with the Artus assay.

Specimen processing and testing
192 plasma samples, taken from January 2013 through July 2013 from adults for routine HIV-1RNA viral load monitoring or diagnostic workup were used in our study. During phase 1 of the study (from April 2013 until June 2013), 157 positive frozen stored aliquots, previously analyzed by the Versant assay were reanalyzed retrospectively with the Artus assay, based on the QIAsymphony RGQ automated assay and according to the manufacturer's instructions. The QIAsymphony system allows the use of primary blood tubes (in an off-label manner) and barcode reading, but in phase 1 secondary tubes were employed. During the second phase (from June 2013 until July 2013), 35 positive and negative clinical specimens (tested with the Versant assay and stored at +4°C for no longer than 24 hours) were reanalyzed with the Artus assay, using the same primary tubes. In this phase, both systems were linked to the Laboratory-Information-System (LIS), and work lists were generated after barcode reading.

Nucleic acid extraction
The Artus assay includes the QIAsymphony SP/AS, an automated instrument for sample preparation (SP) and assay set-up (AS), followed by fluorescence-based real time qPCR and data analysis on the RotorGene Q, QIAGEN's re-

al-time PCR cycler. The Versant assay consists of an automated sample preparation and assay set-up followed by Amplification/Detection (AD) by real-time PCR (Troppan, Stelzl *et al.*, 2009). HIV-1 RNA extraction from plasma was performed by the QIAsymphony SP instrument, using the QIAsymphony DSP Virus/Pathogen Midi Kit. The kit utilizes magnetic-particle technology which enables a nucleic acid isolation and purification. Moreover, to reduce time-consuming manual processes, the reagents used for purification of nucleic acids (magnetic particles, lysis buffer, wash buffer and elution buffer) are contained in a reagent ready-to-use cartridge. The volume of plasma needed for the extraction, including excess volume, is 1200 µL whereas purified RNA is obtained from 1000 µL. An internal control (IC) is supplied with the kit and introduced into the purification procedure to monitor the efficiency of the purification phase as well as possible PCR inhibition. The IC is added with carrier RNA-buffer AVE for a total of 120 µL mixture for each sample, for a final elution volume of 60 µL. Samples processed on the QIAsymphony SP instrument were transferred automatically to the QIAsymphony AS instrument. Briefly, 20 µL of the eluate was transferred to a strip tube containing 30 µL of the master mix, already prepared by the instrument, for a final reaction volume of 50 µL. The master mix contains reagents and enzymes for the reverse transcription (RT) and specific amplification [5′ segment of the long terminal repeat (5'LTR)] of the HIV-1 genome. The Versant assay is also an automated amplification assay, composed of a sample preparation module for RNA extraction and an amplification/detection module for real-time qPCR. For the extraction phase, 650 µL of plasma is needed, of which 500 µL is used in the protocol. All reagents needed for the lysis phase, as well as the IC, are mixed automatically by the instrument. From a total of 70 µL elution volume, 55 µL are transferred into a well of the PCR plate containing 20 µL of the primer/probe mix for a final reaction volume of 75 µL, used for the reverse transcription-qPCR (RT qPCR).

HIV-1 quantification

On the Rotor-Gene Q, reverse transcription and specific amplification were performed imme-diately after extraction, targeting a region of 93 bp located in the unique 5'LTR of the HIV-1 genome. Both the target and the IC were amplified simultaneously and detected in fluorescence channel cycling Green and Orange, respectively. The threshold value for the real time qPCR analysis was set to 0.04 for all runs. To generate a standard curve on Rotor-Gene Q Instrument, the 4 quantification standards (QS 1-4) were treated as previously purified samples, and the same volume was used (20 µl). Furthermore, for detection of PCR contamination, no-template controls (NTCs) were included on each batch of samples, and results were analyzed with Rotor-Gene Q software version 3.1. Conversely, the Versant assay targets region within the HIV-1 integrase section of the *pol* gene. In each PCR plate, two calibrators and three external controls (high and low positives and negative) were used as quantification standards and for control of PCR contamination, respectively. Viral load for both assays was expressed as the number of HIV-1 RNA copies per milliliter. The analytical sensitivity of the Artus assay, using the *artus* HI Virus-1 RG Kit, in combination with the Rotor-Gene Q, is 34.4 copies/ml, whereas the linear range was calculated to cover concentrations from 45 to 4.5 x 10^7 copies/ml. Similarly, the analytical sensitivity of the Versant assay is 37 copies/mL with a linear range from 37 to 1.1×10^7 copies/ml.

RESULTS

A total of 192 routine clinical plasma samples were enrolled in our study (Table 2). Samples were considered "*positive*" if detected above the analytical LOD (>37 copies/ml and >34 copies/ml in the Versant assay and the Artus assay, respectively), "*negative*" if the target was not detected, and as "*low positive*" if they were below the analytical LOD (<37 copies/ml and <34 copies/ml in the Versant assay and the Artus assay, respectively). Three samples (1.55%) were excluded from the study as follows: one sample had a late quantification cycle (Cq) with the Versant assay, and therefore considered to have an invalid result (negative in the Artus assay), whereas for the other two samples amplification was inhibited with the Artus assay (nega-

TABLE 2 - *Results obtained by both assays compared with the Quality Control for Molecular Diagnostics (QCMD) 2012 HIVRNA12A proficiency panel - External quality assessment.*

HIVRNA12A QCMD Panel (genotype)	QCMD Target (c/ml)	Siemens (c/ml)	QIAGEN (c/ml)	QCMD Target (log c/ml)	Siemens (log c/ml)	delta QCMD	QIAGEN (log c/ml)	delta QCMD
1 (B)	3475	3337	1422	3.54	3.52	-0.02	3.15	-0.39
2 (B)	1315	1295	752	3.12	3.11	-0.01	2.88	-0.24
3 (C)	944	610	117	2.97	2.79	-0.19	2.07	-0.91
4 (B)	3475	3200	1696	3.54	3.51	-0.04	3.23	-0.31
5 (B)	237	252	27	2.37	2.40	0.03	1.43	-0.94
6 (A/G)	34914	3028	27403	4.54	3.48	-1.06	4.44	-0.11
7	0	0	0	0.00	0.00	0.00	0.00	0.00
8 (C)	10162	6824	1992	4.01	3.83	-0.17	3.30	-0.71
Mean Log$_{10}$ difference						**-0.18**		**-0.45**

tive and 32 copies/ml in the Versant assay). Of the remaining 189 samples, 130 samples had positive results and 18 samples were detected as negative by both assays. The median log$_{10}$ difference between the positive results, obtained by the Artus assay and the Versant assay was -0.01 log$_{10}$.

One hundred and six (81.5%) samples were found to be within ±0.5 log$_{10}$, 21 (16.2%) samples were found to be within ±0.51 log$_{10}$ and ±0.99 log$_{10}$, whereas only three (2.3%) samples had >1.0 log$_{10}$ deviation (Figure 1). Among the 130 positive samples detected by both assays,

74 (57%) samples had higher results obtained by the Artus assay (median log$_{10}$ difference of -0.31), whereas 56 (43%) samples had higher results obtained by the Versant assay (median log$_{10}$ difference of 0.37). Thus, considering the 130 positive samples (by both assays), highly correlated results were obtained, as shown by the XY scatter plot (R^2=0.858, Figure 1) and by the Bland.Altman analysis (Figure 2).

In samples with low positive results no correlation was observed (R^2=0.09). In fact, of the four samples detected as low positive by the Versant assay (median 22 copies/ml; range 12-35 cop-

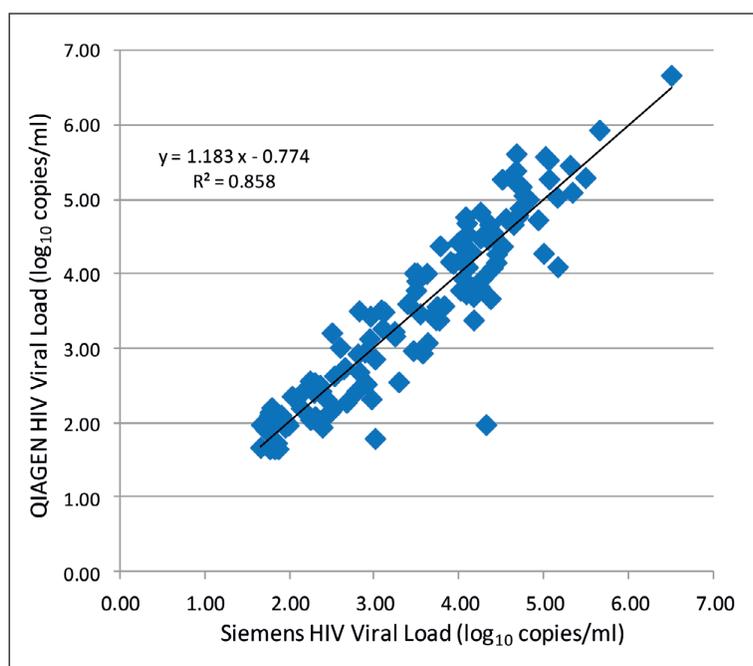$$y = 1.183\,x - 0.774$$
$$R^2 = 0.858$$

FIGURE 1 - *Logarithmic linear correlation between results obtained with the Artus assay and the Versant assay for 130 positive samples. Only samples above the lower linear range, quantified by both assays, were included in the analysis. Discrepant samples and those below LOD in both assays excluded from the Bland-Altman analysis were as follow: eight samples tested positive only with the Versant assay, with viral load slightly above the assay lower LOD (median 57 copies/ml; range 44-74 copies/ml); 29 samples, quantified with the Artus assay as low positive were detected above the Versant lower LOD (median 80 copies/ml; range 40-201 copies/ml), whereas, four samples detected as low positive by the Versant assay (median 22 copies/ml; range 12-35 copies/ml) of which, 3 were detected as negative and one as low positive (7 copies/ml) by the Artus assay.*
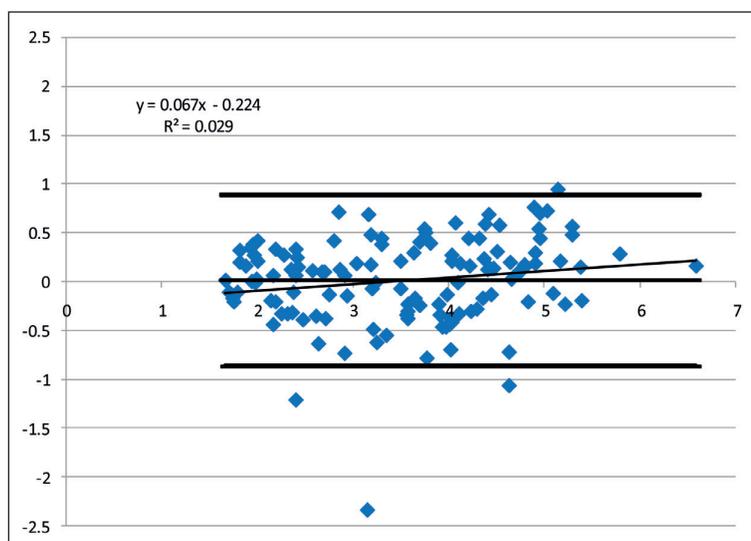
FIGURE 2 - *Bland-Altman analysis of the agreement between Artus assay and Versant assay. The x-axis indicates the mean values for each sample by the two assays. The y-axis indicates the differences between the values obtained by the two methods. The solid line represents the mean difference between the values, and the dotted lines represent the mean difference ± 1.96 SD (95% limits of agreement). Only samples above the lower linear range, quantified by both assays, were included in the analysis. The three most discrepant samples (>1 Log_{10}) were genotyped and resulted as genotype A (two of them; log differences -1.21 and -2.34) or genotype C (log difference -1.06).*

ies/ml), three were detected as negative and one as low positive (7 copies/ml) by the Artus assay; whereas, 29 samples, quantified with the Artus assay as low positive were detected as positive by the Versant assay (median 80 copies/ml; range 40-201 copies/ml). The remaining eight (4.18%) samples were quantified only by the Versant assay, with median of 55 copies/ml (range 42-74 copies/ml), and were not detected by the Artus assay.

Results from phase 1 (157 samples, tested with the Versant assay and stored at -80°C, before retested with the Artus assay) and from phase 2 (35 fresh samples, analyzed with the Versant assay, stored at +4°C for no longer than 24 hours and reanalyzed by the Artus assay), are shown in Table 3. In phase 1, the Artus assay detected 25 samples as low positive (median 15 copies/ml) against only one low positive sample detected by the Versant assay (14 copies/ml), with median $\log_{10}$ difference in these samples of 0.80.

Moreover, 7 samples were detected as negative, by the Artus assay, where all of them were de-

TABLE 3 - *Summary of all samples enrolled in the study.*

Samples (enrolled)	Assay		Phase I		Phase II	
	Versant	Artus	Versant	Artus	Versant	Artus
	HIV-1 RNA 1.0	HI Virus-1 QS-RGQ	HIV-1 RNA 1.0	HI Virus-1 QS-RGQ	HIV-1 RNA 1.0	HI Virus-1 QS-RGQ
Total of samples (192)	192	192	157	157	35	35
Amplification inhibition	1	2	1	0	0	2
Samples enrolled (189)	—	—	—	—	—	—
Positive samples (171)	171	160	156	149	15	11
- Above analytical sensitivity	167	130	155	124	12	6
- Low Positive	4	30	1	25	3	5
- Negative	0	11	0	7	0	4
Negative samples (18)	18	18	0	0	18	18
Discrepant samples						
Positive	8	0	7	0	1	0
Negative	0	8	0	7	0	1
QCMD 2012 HIVRNA12						
Positive (7)	7	7				
Negative (1)	1	1				

Quality Control for Molecular Diagnostics (QCMD) 2012 HIVRNA12A proficiency panel. *Low positive* - Samples detected below the assay's analytical sensitivity. Phase I - 157 positive frozen stored aliquots, previously analyzed by the Versant assay, were reanalyzed retrospectively with the Artus assay. Phase II - 35 fresh positive and negative samples.

tected slightly above the LOD by the Versant assay (median 51 copies/mL). In phase 2, however, 18 samples were detected as negative by both assays; while 15 of the remaining samples were detected as follow: 5 and 3 samples as low positive, 6 and 12 as positive and 4 and 0 samples as negative by the Artus assay and the Versant assay, respectively. Importantly, the three samples excluded from the study, due to inhibition or late Cq, were all fresh samples (one sample in phase 1 with the Versant assay and two samples in phase 2 with the Artus assay).

To ensure qualitative and quantitative analysis accuracy, an external quality assessment was used. For each assay, a Quality Control for Molecular Diagnostics (QCMD) 2012 HIVRNA12A proficiency panel (8 samples), that contains group B, C and an A/G subtypes, was processed, and the results were compared (Table 2). All seven positive samples from the QCMD 2012 HIVRNA12A panel were detected as positive by both assays (median $\log_{10}$ differences of -0.45 $\log_{10}$ and -0.18 $\log_{10}$, respectively), and the negative sample was found to be negative by both assays.

DISCUSSION

This study evaluated the performance of the *artus* HIV-1 QS-RGQ assay using the QIAsymphony RGQ system on routine clinical samples taken from adults for both diagnosis and therapeutic monitoring. Results were compared to those obtained by the routine laboratory assay - Versant assay.

Both platforms are highly automated systems, which consist of primary sample loading, barcode sample identification, automated liquid handling, extraction and processing, as well as qPCR integration. These characteristics are now considered a requirement for most diagnostic laboratories, especially for viral quantification assays. For this reason, we used the full automated QIAGEN system, in the novel configuration, which includes the QIAsymphony SP/AS instrument for sample preparation and assay set-up, and the RotorGene Q real-time PCR cycler for reverse transcription and target amplification. Overall, 148 (77.5%) samples (130 positive, above the linear range of detec-

tion, and 18 negative) were detected by both assays. The Artus assay showed a sensitivity of over 93% on samples previously tested with the Versant assay. For these samples, both assays demonstrated a similar performance, with highly correlated results, as shown by the XY scatter plot (R^2=0.858, Figure 1). Bland-Altman analysis (Figure 2) also demonstrates that the difference between the two assays was low, and that overall, the Artus assay did not differ significantly from the Versant assay. Only eight (4.2%) samples (7 samples from phase 1 and only one sample from phase 2), had discrepant results and tested positive only with the Versant assay, with viral load slightly above the lower LOD (median 57 copies/ml; range 44-74 copies/ml), whereas, they tested as negative with the Artus assay.

As stated by the manufacturers, the lower limit of the Artus assay's linear range (45 to 4.5 x 10^7 copies/ml) is slightly higher than that of the Versant assay (37 to 1.1×10^7 copies/ml), whereas the analytical LOD is equal in both assays (34.4 and 37 copies/ml, respectively). Unfortunately, RNA stability in terms of sample storage conditions is still an open question, especially on low and undetectable viral loads. Recently, data from a large international study confirmed the observation of a decreased correlation between different assays, at low viral loads that are close to the LOD of the assays.

Although most newer assays may detect even very low number of viral RNA particles, preanalytical variables and their influence on the quality of assay results, as well as assay reproducibility, should be taken into consideration (Swenson, Cobb *et al.*, 2014). Although some studies conclude that HIV-RNA quantification did not differ in plasma stored at different temperatures (Bonner, Siemieniuk *et al.*, 2014), we cannot exclude that sample storage at -80°C (phase 1) until re-quantification with the Artus assay, may have had an impact on the results. In fact, in our study, only 35 fresh plasma samples were tested prospectively (phase 2) with both assays and therefore, correlation analysis for phase 2 could not be reliably performed, due to the low number of samples. The lower limit of the Artus assay's linear range, recently reduced by the manufacturer (from 112 copies/ml to 45 copies/ml), and supported by previous

comparison with the Abbott RealTime HIV assay and with the Roche CCOBAS AmpliPrep/ COBAS TaqMan HIV-1 test v2.0 (Drexler, Reber et al. 2012; Wall, Perinpanathan *et al.,* 2012) is slightly higher than that of the Versant assay (37 copies/ml), even if the analytical LOD is equal in both assays (34.4 and 37 copies/ml, respectively).

Our data, even if with the above-mentioned biases, support previous observations showing increasing discrepancy among different HIV-1 viral load assays when viremia approaches the LOD (Yan, Hanafi *et al*. 2010; Swenson, Dong *et al*. 2013). Another limitation of our study could be that even if more than 80% of patients routinely tested in our laboratory are infected by HIV-1 subtype B viruses (Gianotti, Galli *et al.,* 2013), we could not establish the genotype of all discordant samples due to lack of residual material and low viremia.

However, the three most discrepant samples (>1 Log_{10} difference) among those amplified by both assays and within the linear range were genotyped as previously described (Gianotti, Galli *et al.,* 2013), and resulted as genotype A (two of them) or genotype C. To further evaluate the QIAsymphony RGQ system, we tested an external control panel [Quality Control for Molecular Diagnostics (QCMD) 2012 HIVRNA12A proficiency panel] that contains group B, C and an A/G subtypes. When results from each assay were compared to the expected QCMD panel quantification (Table 2), the mean Log variation was similar with the two assays (-0.18 and -0.45 for the Versant and the Artus assays, respectively) and discrepancies were observed not only with non-B subtypes. However, due to the low number of HIV-1 subtypes in the QCMD12A panel, and in our patient population, an extended number of non-B subtypes should be included for future evaluations.

Moreover, the results of the *artus* HIV-1 QS-RGQ were lower for all samples when compared to the Siemens assay. In conclusion, based on the above results, we believe that the quantitative *artus* HIV-1 QS-RGQ CE-IVD kit, together with the QIAsymphony RGQ system complete workflow, can be considered a potential clinical tool for the routine detection and quantification of HIV-1 RNA in plasma.

Abbreviations used in this article
Human immunodeficiency virus type 1 (HIV-1), quantitative real-time polymerase chain reaction (qPCR), limit of detection (LOD), antiretroviral therapy (ATR), sample preparation (SP), assay setup (AS), internal control (IC), quantification cycle (Cq), Quality Control for Molecular Diagnostics (QCMD), long terminal repeat (LTR), no-template control (NTC), reverse transcription-qPCR (RT qPCR), Laboratory-Information-System (LIS).

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