Evaluation of the *artus*® CMV QS-RGQ assay for automated extraction, assay setup, and detection of CMV DNA in clinical respiratory and urine samples

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QIAsymphony® RGQ, in combination with the *artus* CMV QS-RGQ assay, is highly suited for the extraction and detection of CMV DNA in clinical urine and respiratory samples.

Introduction

Accurate measurement of human cytomegalovirus (CMV) DNA titers in peripheral blood is a standard of care for recipients of both solid organ and stem cell transplants. Reactivation of latent CMV, or infection via the graft, can cause serious morbidity and mortality in this patient group. Detection and quantification of CMV DNA can be used as an indicator to begin antiviral treatment to reduce CMV replication and related CMV disease (1, 2).

In addition to replication and detection in peripheral blood, CMV can also be a causative agent of pneumonitis, primarily in immunocompromised individuals. Furthermore, CMV can be congenitally acquired, causing abnormalities in the newborn child and, in the most extreme cases, death (3).

A differential diagnosis is often required in these clinical presentations in order for the correct clinical management to be implemented (1–3). For this reason, we conducted a study to evaluate the usefulness of the QIAsymphony RGQ in combination with the *artus* CMV QS-RGQ assay to detect CMV DNA in both urine and broncheo-alveolar lavage (BAL) samples (application not CE-IVD–marked).

Materials and methods

The QIAsymphony RGQ system was used to process 25 BAL and 40 urine samples that had previously been tested with a laboratory-developed assay at Public Health England (PHE) Cambridge. For this study, all BAL and urine samples were retrieved from –70°C storage. BAL samples were processed by mixing 400 µl of sample with 100 µl of Mucolyse (Pro-Lab Diagnostics, Inc.) in a 2 ml microcentrifuge tube, followed by a brief vortex, and then incubated in a heating block at 56°C for 30 minutes. This was performed in a Category III facility before being transferred back to the Category II laboratory for nucleic acid purification. No pre-processing of the urine samples was performed. ▶

The QIAsymphony RGQ. A modular system that can maximize your efficiency and optimize workflows, from sample to result.

The *artus* CMV QS-RGQ Kit. An automated workflow, from sample to CMV detection, using the QIAsymphony RGQ.

To establish an estimated sensitivity level in urine, the WHO International Standard for human cytomegalovirus (NIBSC, UK REF 09/162) was resuspended in molecular biology grade water (as per instructions) and a dilution series (500,000 to 50 IU/ml) was prepared with CMV DNA-negative urine.

QIAsymphony SP DNA extraction

Both urine and BAL samples were processed on the QIAsymphony SP with the QIAsymphony DSP Virus/Pathogen Midi Kit. The Complex400 DSP protocol was used with an input volume of 400 µl and an elution volume of 60 µl. Samples were spiked with *artus* CMV internal control during QIAsymphony SP processing to monitor extraction efficacy and possible PCR inhibition.

QIAsymphony assay setup and amplification

Samples were transferred to the QIAsymphony AS for assay setup with 10 µl of purified eluate/control added (by the system) to 15 µl of mastermix (CMV RG Master) in 0.1 ml strip tubes. The tubes were sealed, transferred to the Rotor-Gene® Q system, and the PCR assay started. Samples were analyzed and reported in copies/ml of original sample for urine, or as a qualitative result for BAL samples. The *artus* CMV QS-RGQ assay targets the major immediate early (MIE) gene and has a limit of detection (LOD) of 43 copies/ml in plasma.

PHE Cambridge in-house workflow

The laboratory-developed assay, targeting the CMV DNA polymerase gene, is based on nucleic acid extraction procedures using the QIAGEN® BioRobot® MDx system for urine samples (300 µl input/80 µl output, QIAamp® One-for-All Nucleic Acid Kit) and the bioMérieux® NucliSENS® easyMAG® system for BAL samples (200 µl input/80 µl output, generic 2.0.1 protocol), followed by assay setup on the QIAgility® platform (10 µl sample eluate/control and 20 µl mastermix) and real-time quantitative PCR (qPCR) performed on the Applied Biosystems® 7500 Real-Time PCR System. Prior to loading onto the easyMAG system, 200 µl of the processed BAL sample was added to 200 µl of easyMAG lysis buffer in a 2 ml microcentrifuge tube, vortexed, and incubated for 10 minutes at 56°C. Quantitation was achieved by using external plasmid standards to produce a calibration curve for determination of the viral load in each of the clinical samples. Results are reported in copies/ml of original sample for the urine samples, and as a qualitative result (CMV DNA detected or CMV DNA not detected) for the BAL samples. All samples were spiked with a murine CMV internal control to check for extraction efficacy and possible PCR inhibition. The assay has a limit of detection (LOD) of 300 copies/ml in plasma.

Results

Results for urine samples were analyzed and transformed into log values for comparison in an XY scatter plot and Bland-Altman analysis. The BAL sample results were recorded in a qualitative manner and are presented in a bar graph format.

Urine samples

In both methods (PHE Cambridge in-house workflow and QIAsymphony RGQ), 9/40 (23%) urine samples were negative and 31/40 (77%) were positive. A viral load of $>10^{10}$ copies/ml was observed in 2/40 (5%) urine samples upon initial testing, so these samples were removed from further analysis because they exceeded the linear ranges of both the *artus* CMV QS-RGQ and PHE Cambridge assays. Based on these results, both assays were 100% concordant.

The mean log difference in urine samples was –0.51 $\log_{10}$ copies/ml (QIAGEN – PHE). The XY scatter plot (Figure 1) shows how both assays compare in positive samples, and the Bland-Altman plot (Figure 2) shows the agreement between both assays, with just 1/31 (3%) positive samples outside the +/– 2 standard deviation (SD) range. The data shows that the assays compare well and are in agreement.

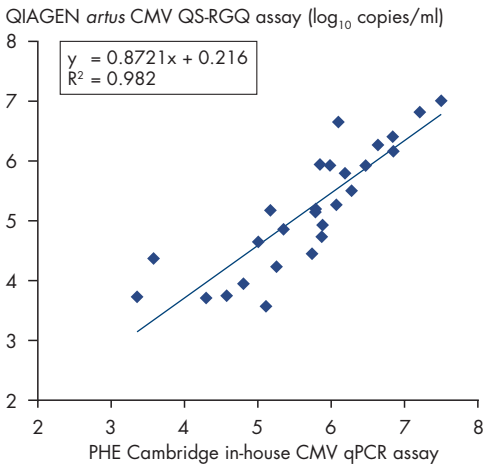

Figure 1. XY scatter plot. Comparison of PHE Cambridge in-house assay and *artus* CMV QS-RGQ assay for CMV-positive urine samples.

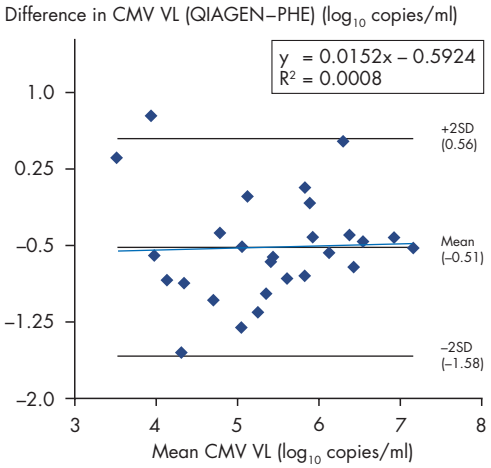

Figure 2. Bland-Altman plot. Agreement between PHE Cambridge in-house assay and *artus* CMV QS-RGQ assay.

The WHO International Standard was diluted in CMV-negative urine and tested in single replicates. Based on this initial dilution series, CMV DNA in urine can be detected as low as 500 IU/ml when using the *artus* CMV QS-RGQ assay, demonstrating good sensitivity (Table 1). Additional experiments are required to determine the true analytical sensitivity using hit rate and probit analyses.

Table 1. WHO International Standard for human cytomegalovirus dilutions

Target viral load (IU/ml)	Target viral load (log IU/ml)	*artus* CMV copies/ml	*artus* CMV log copies/ml
500000	5.70	73,964	4.86
50000	4.70	6,723	3.82
5000	3.70	719	2.85
500	2.70	58	1.76
50	1.70	0	0

BAL samples

In both methods (PHE Cambridge in-house workflow and QIAsymphony RGQ), 9/25 (36%) BAL samples were negative and 15/25 (60%) were positive. A discordant result was observed in 1 (4%) BAL sample (C_T value of 39 using the PHE Cambridge in-house assay and CMV DNA not detected by the *artus* CMV QS-RGQ assay). It is likely that the very low viral load in this sample (reflected by the late C_T value) and freeze/thawing affected the ability of the *artus* CMV QS-RGQ assay to detect any CMV DNA. The concordance in BAL samples is therefore 96%. Qualitative results are shown in the bar chart below (Figure 3). The mean C_T value difference of the positive samples was –3 C_T (QIAGEN – PHE).

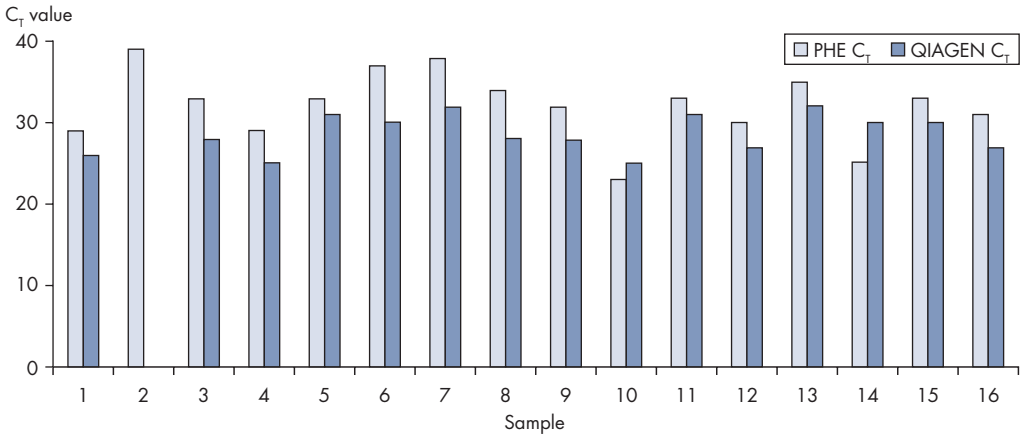

Figure 3. Qualitative results. C_T values for 16 BAL samples using the PHE Cambridge in-house assay and *artus* CMV QS-RGQ assay.

Conclusions

- This study shows that QIAsymphony RGQ, in combination with the *artus* CMV QS-RGQ assay, can be used for extraction of CMV DNA from urine and BAL samples, and for detection of CMV DNA in diverse and complex clinical samples.
- High concordance was observed for both urine (100%) and BAL (96%) samples. Only one late positive BAL sample was not detected when retested with the *artus* CMV QS-RGQ system.
- The QIAsymphony RGQ platform resulted in C_T values that were 3 cycles earlier on average, which demonstrates the superior DNA purification and elution capabilities of the QIAsymphony SP compared to the NucliSENS easyMAG.

References

1. Kotton, C.N. et al. (2010) International consensus guidelines on the management of cytomegalovirus in solid organ transplantation. Transplantation **89**, 779.
2. Kotton, C.N. (2010) Management of cytomegalovirus infection in solid organ transplantation. Nat. Rev. Nephrol. **6**, 711.
3. Atkinson, C. and Emery, V.C. (2011) Cytomegalovirus quantification: Where to next in optimising patient management? J. Clin. Virol. **51**, 223.

Ordering Information

Product	Contents	Cat. no.
QIAsymphony RGQ, system	QIAsymphony SP, QIAsymphony AS, Rotor-Gene Q 5plex HRM; includes required accessories and consumables, installation, and training; includes 1-year warranty on parts and labor	Inquire
QIAsymphony DSP Virus/ Pathogen Midi Kit	For 96 preps (1000 µl each): includes 2 reagent cartridges and enzyme racks and accessories	937055
artus CMV QS-RGQ Kit (24) CE	For 24 reactions on the QIAsymphony RGQ: Master, Mg Solution, 4 Quantitation Standards, Internal Control, Water (PCR grade)	4503363

The presented application using the QIAsymphony DSP Virus/Pathogen Midi Kit and *artus* CMV QS-RGQ Kit (24) CE, in combination with the QIAsymphony RGQ, is for Research Use Only. Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

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