

# Comparison of workflow and data analysis for the quantification of Cytomegalovirus (CMV) using an in house method versus QIA Symphony® RGQ system.

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## Background and Aims

Cytomegalovirus (CMV) infection is a significant cause of morbidity and mortality amongst transplant recipients and other immunocompromised patients<sup>1</sup>. Ongoing monitoring of these patients is an important tool to detect and treat CMV viraemia, before the onset of disease. Around 15,000 quantitative CMV PCRs are performed annually at the Royal Free and are generated using an in-house assay with a semi-automated nucleic acid extraction. The new *artus* CMV QS-RGQ integrated assay protocol offers an automated solution to viral load determination. The workflow and data analyses generated with the new system were compared to the in-house quantitative PCR method.

## Methods

CMV viral loads were determined using an in-house realtime PCR method<sup>2</sup> and the Qiagen *artus* QS-RGQ CMV kit. Both use specific primer and probe sequences targeting UL55 and UL123 respectively to allow for viral DNA amplification and detect fluorescent probe molecule as a measure of successful amplification.

Batches of 4.0ml citrated blood samples for CMV testing were prepared within the routine testing laboratory. All procedures were performed by a single member of staff who followed a standard operating procedure. For each batch the tasks performed, and accurate timing data was recorded by a secondary observer. In total six batches were performed by two separate operators who were either junior or experienced staff with the prior knowledge of the procedure.

For the QS-RGQ *artus* assay whole citrated bloods were batched into groups of 19 following routine diagnostic testing. Six integrated assays were performed on the QIA Symphony according to the manufacturer's instructions, utilising full barcode scanning, sample tracking, extraction and assay setup. Real-time PCR tubes were amplified using RotorGene Q thermal cycler (RGQ) and data analysed either by the standard RotorGene Q software or a pre-release copy of RotorGene Q AssayManager.

To allow inter-assay comparisons whole citrated blood samples were re-tested using the *artus* CMV assay and compared against the in-house diagnostic assay. In addition, a serial dilution of the 1st WHO CMV international standard (NIBSC) in triplicate was performed with both assays.

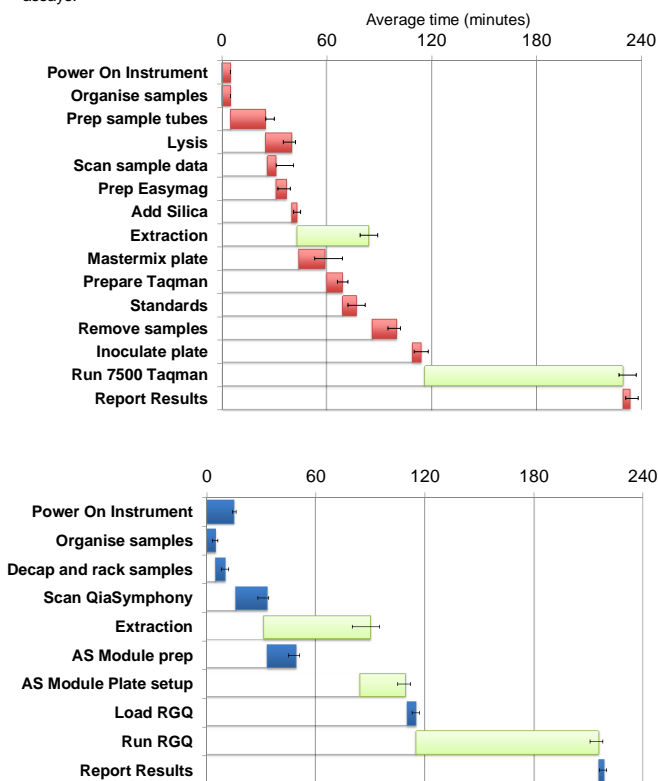


Figure 1. Time comparison of tasks required to complete quantitative realtime PCR CMV DNA viral load assay in whole citrated blood. In-house assay performed by Royal Free Virology department (top) with tasks (red) and hands-free time (green). Qiagen QS-RGQ *artus* CMV kit assay with integrated assay setup showing tasks (blue) and hands-free time (green).

## Results

Times for all six runs were averaged for each workflow according to the major tasks involved (Figure 1). The total time required for the in-house workflow ranged from 215 to 250 minutes (mean 232).

For the QS-RGQ runs analysed with the standard computer software the total times ranged from 225 to 241 minutes (mean 233). When the assay was analysed using the pre-release version of the fully automated analysis software (RotorGene Q AssayManager) the time was reduced to 214 to 218 (mean 216). The overall average total time for the QS-RGQ assay was therefore 220 minutes.

Total time taken when using QS-RGQ *artus* CMV kit was reduced by 12±6 minutes.

The average hands-off time for the user (where no tasks were required to complete the assay) increased (by 62±3 minutes) using the QIA Symphony-RGQ system (Figure 1). There was also a high level of concordance between in-house laboratory generated data with those from the QIA Symphony-RGQ (Figure 2A). Nineteen samples were run in duplicate on both assays. The data generated by the new system provided results in copies/ml that were equivalent to the international standard in IU/ml (average deviation from the mean log<sub>10</sub> -0.04 (Figure 2B).

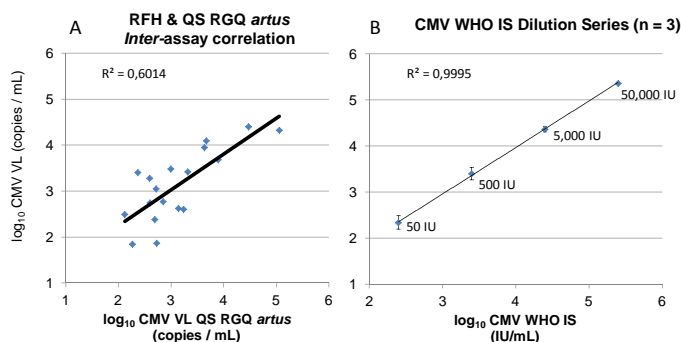


Figure 2. Comparisons of CMV viral loads generated using in-house and QS-RGQ *artus* assays. (A) Correlation of log<sub>10</sub> viral loads from 19 citrated blood samples in duplicate. (B) Comparison of viral loads determined from serial dilution of 1<sup>st</sup> WHO International standard. Points are labelled with number of international units per reaction.

## Conclusions

Despite only moderate reduction in the overall time taken to complete the assay, the *artus* CMV QS-RGQ assay provides a substantial increase to the hands-off time to allow the user to undertake other duties. This in turn leads to increased laboratory efficiency and cost savings by freeing up a member of staff to complete other tasks in improved time.

The *artus* CMV QS-RGQ integrated assay protocol offers an efficient and accurate alternative to current in-house protocols for quantitation of CMV in whole blood. The automated extraction and assay setup platform also reduces the likelihood of introducing human error during the processing tasks and reduces intra-assay variability. A major time saving was made by introducing automated assay analysis by the RotorGene Q AssayManager software. Prior to this each sample was inputted manually and viral loads were calculated by introducing additional run data. The use of AssayManager also prevented transcription errors.

This study showed good agreement and correlation of data using both current in-house protocols and the automated system. The strength of the correlation was greater at viral loads more than one log greater than the limit of quantification (200 copies/ml). Viral loads around the LOQ were seen to display an expected level of stochastic variation. In conclusion, the QIA Symphony® RGQ system is a versatile platform that is able to provide numerous improvements in sample handling efficiency and accuracy within a routine virology laboratory setting.

## Summary

- Time to complete QS-RGQ *artus* CMV assay reduced by 12±6 minutes but hands-off time increased by 62±3 minutes vs. in-house.
- The fully automated system also improves quality assurance, reduces opportunity to introduce error and maintains intra-assay consistency.

## References

1. Ljungman P, Griffiths P, Paya C. Definitions of Cytomegalovirus Infection and Disease in Transplant Recipients. *Clin Infect Dis*. 2002 Apr 15;34(8):1094-7.
2. Atkinson C, Griffiths P, et al. Use of Stored Dried Blood Spots for Retrospective Diagnosis of Congenital CMV. *Journal of Medical Virology* 81, no. 8 (2009): 1394-1398.



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