CMV R-gene™ real time PCR assay and the candidate 1st WHO International Standard for Human Cytomegalovirus : Standardization of nucleic acid amplification techniques

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Introduction

The human cytomegalovirus (HCMV) is an ubiquitous herpes virus with a high prevalence worldwide. It causes disease in the immunologically-naïve, such as new-borns and infants, and immunosuppressed individuals, particularly transplant recipients and AIDS patients. Severe and life-threatening HCMV infections in immunocompromised individuals are managed through the administration of anti-herpetic agents, however, all are associated with toxicity during prolonged use.
Consensus guidelines for the management of HCMV infection and disease recommend the use of NAT-based approaches in order to determine viral load measurements in pre-emptive programs for disease prevention.
The CMV R-gene™ kit is validated on the major extraction and real time PCR platforms as well as different sample types like plasma, whole blood, …
The lack of an available standardised reference system for HCMV makes it difficult to compare viral load measurements between assays/laboratories and to define a uniform policy for the management of therapeutics (cut-off, follow-up..). The 1st WHO International Standard for HCMV was developed by NIBSC (code 09/162). The candidate standard is established as the International Standard for HCMV with an assigned potency of $5x10^6$ International Units (IU).
CMV positive control was developed in parallel to check the intra-laboratory variability and demonstrate the stability of results between two calibrations with WHO standard.
The aim of this study is to evaluate CMV R-gene™ kit quantification with the HCMV International Standard and to present the first results with CMV positive control r-gene™.

Materials and Methods

Amplifications: 10µL of extracted sample were added to 15µL of ready-to-use CMV R-gene™ (69-003) amplification premix.
HCMV WHO CMV R-gene™ results and Conversion factor (copies/mL IU/mL) was obtained using eight results: one dilution (10 fold) in plasma of HCMV WHO containing 5.10^6 UI/mL was tested in duplicate in 4 independent assays (extraction and amplification). The experiment was performed on ABI 7500Fast (Applied Biosystems), Rotorgene 6000 (Corbett), LC480 (Roche), Dx Real Time System (Bio-Rad) after NucliSENS® easyMAG® (bioMerieux) extraction, or with Versant kPCR Molecular System (Siemens).
Conversion factor used with CMV R-gene™ assay: a sample containing 1.00E+04 copies/mL, diluted 10 fold in plasma or whole blood, has been evaluated. The experiment was performed on ABI 7500Fast, Rotorgene 6000, LC480, Dx Real Time System, after easyMAG® NucliSENS® extraction, or with Versant kPCR Molecular System.
CMV positive control (ref. 68-015) was systematically tested as a whole blood sample.

Results

HCMV WHO with CMV R-gene™ kit quantification

The conversion factor is obtained as follow :

$$\frac{\textit{HCMV WHO Quantification (IU/mL)}}{\textit{Mean of Argene Quantification (copies/mL)}}$$

Conversion for plasma on different extraction and amplification platforms

Matrix	Extraction platforms	Amplification platforms	Mean of Argene Quantification (copie/mL)	HCMV WHO Quantification (IU/mL)	Conversion Factor
Plasma	easyMAG®	ABI 7500Fast	1.74E+06	5.00E+05	0.288
		Rotorgene 6000	1.89E+06		0.265
		Mx3005P/Versant kPCR AD	1.37E+06		0.365
		Dx Real Time System	1.47E+06		0.340
		LC480	1.75E+06		0.286
	Versant kPCR SP	Versant kPCR AD	4.90E+05		1.020

Conversion factors range from 0.265 to 1.020 depending on the extraction/amplification platforms.

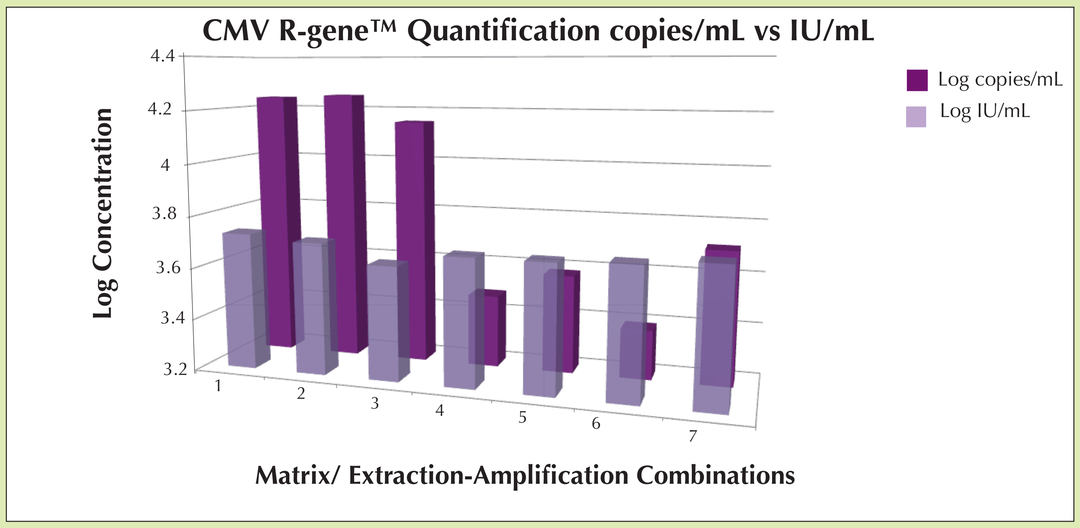
With easyMAG® extraction on plasma, we can obtain a unique conversion factor combined with five amplification platforms: 0.308

Application of conversion factors with different matrix, extraction and amplification platforms

Matrix	Extraction platforms	Amplification platforms	Conversion Factor	Mean of Argene Quantification (copie/mL)	HCMV WHO Quantification (IU/mL)
Plasma	EasyMAG®	ABI 7500Fast (1)	0.308	4.24	3.73
		LC480 (2)		4.25	3.71
		Dx Real Time System (3)		4.15	3.64
Whole blood	EasyMAG®	ABI 7500Fast (4)	1.623	3.48	3.69
		LC480 (5)	1.309	3.58	3.69
		Dx Real Time System (6)	2.016	3.40	3.70
Plasma	Versant kPCR SP	Versant kPCR AD (7)	1.020	3.71	3.72

Deviation standard	0.37	0.03
Coefficient of variation	9.77%	0.80%

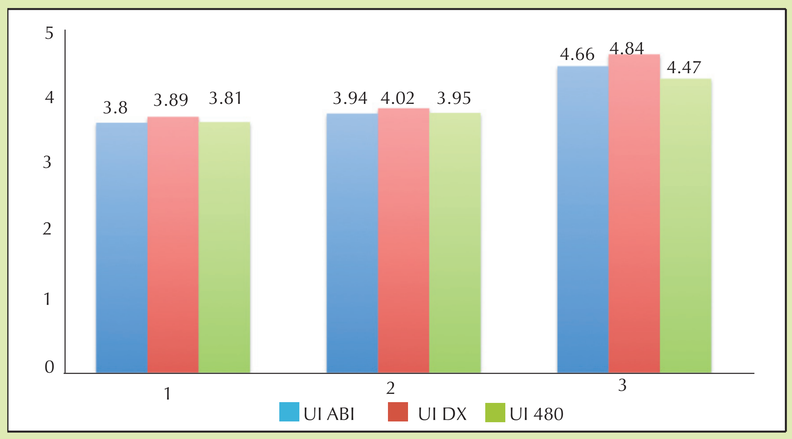
Due to the yield variation between extraction platforms and matrix, different conversion factors must be applied.



The HCMV WHO standard allows to all quantifications values to be range between 3.64 and 3.73, with a coefficient of variation of 0.8%, demonstrating the usefulness of standardization.

CMV Positive control r-gene™ as an external run control QC

Choice of conversion factor: CMV positive control is in a plasma matrix. Most samples used for CMV quantification are whole blood. To determine which conversion factor is to be used, plasma (2) or whole blood (3) conversion factors were applied on positive control quantification (log) after easyMAG® extraction (Whole blood protocol) and amplification on three different platforms. Results are compared to the plasma conversion factor applied after easyMAG® extraction (plasma protocol) (1).



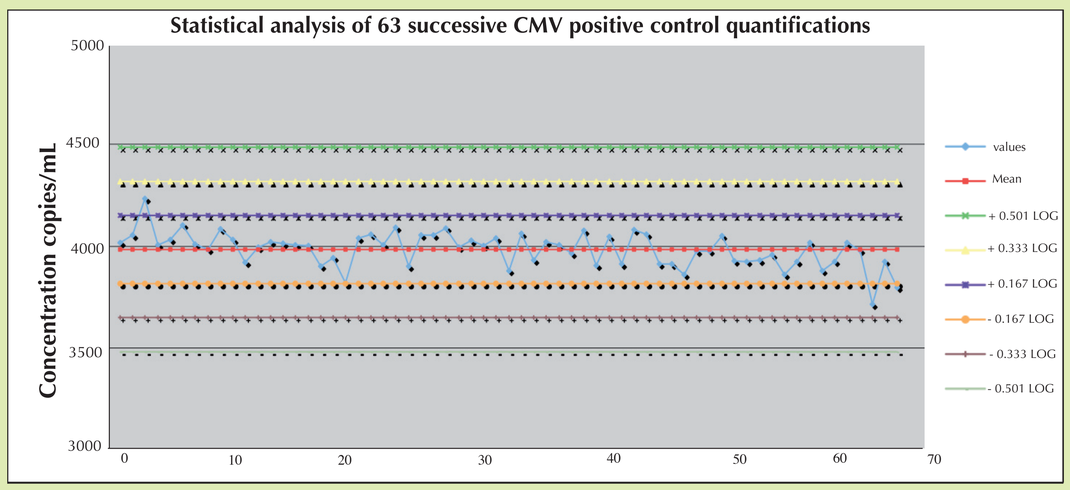
1-Reference plasma extraction protocol and plasma conversion factor

2-Whole blood extraction protocol, plasma conversion factor

3-Whole blood extraction protocol, Whole blood conversion factor

Results clearly show that plasma conversion factor is to be applied, matrix has more effects than extraction protocol on NucliSENS® easyMAG®.

Results of 12 successive experiments on ABI instrument (1 to 19 replicates) were analyzed.



All values (except two) do not exceed 0.16 log shift from the mean value showing the reproducibility of the technique

Conclusions

Quantifications in IU obtained after plasma extraction using easyMAG® and five amplification platforms present non significative variation against each other. A single conversion factor can be applied regardless of the amplification platforms used. With the Versant kPCR complete system, another conversion factor can be used for plasma. As shown on the CMV positive control, matrix has more effects on correction factor than extraction protocol probably as long as adapted to the matrix.
Due to the yield variation of nucleic acid extraction between major extraction systems and sample type (plasma, whole blood), a unique factor can not be determined.
Moreover, the HCMV WHO International Standard allows the comparison of quantification, regardless of the instrument and sample matrix combination.
The use of the CMV positive control as a run control provides a new usefull tool to check stability of the technique and instruments over time.