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

M. VIGNOLES, M. BERTRAND, S. MAGRO, C. BARRANGER. and M. JOANNES.
ARGENE, Parc Technologique Delta Sud, 09340 VERNIOLLE, France
e-mail: matthieu.vignoles@argene.com

Introduction

The human cytomegalovirus (HCMV) is an ubiquitous herpesvirus with a high prevalence worldwilde. It causes disease in the immunologically-naïve, such as newborns 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 with 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).

The aim of this study is to evaluate CMV R-gene™ kit quantification with the HCMV International Standard on the recommanded PCR platforms and sample types described in the IFU.

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 IU/mL was tested in duplicate in 4 independent assays (extraction and amplification). The experiment was performed on ABI 7500Fast (Applied Biosystems), Rotorgene 6000 (Qiagen), LC480 (Roche), Dx real time system (Bio-Rad) after NucliSENS® easyMAG™ (bioMerieux) extraction, or with Versant kPCR Molecular Complete System (Siemens).

Conversion factor used with the CMV Virus R-gene™ assay. A sample containing 1.00E+05 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 Complete System.

Results

HCMV WHO CMV R-gene™ results and Conversion factors calculation (copies/mL -> IU/mL)

Matrix	Extraction Platforms	Amplification Platforms	Argene Quantification (log copies/mL)	Mean of Argene Quantification (log copies/mL)	Standard deviation	Coefficient of variation %
Plasma	EasyMAG™	ABI7500Fast	6.44	6.23	0.10	1.68
			6.25			
			6.15			
			6.06			
			6.28			
			6.18			
			6.27			
			6.19			
		Rotorgene6000	6.35	6.27	0.06	0.88
			6.29			
			6.18			
			6.24			
			6.33			
			6.31			
			6.24			
			6.25			
		Mx3005P/ Versant kPCR AD	6.35	6.11	0.15	2.45
			6.25			
			5.95			
			5.89			
			6.11			
			6.09			
			6.19			
			6.08			
		Dx Real Time System	6.15	6.16	0.07	1.09
			6.18			
			6.23			
			6.28			
			6.07			
			6.09			
			6.16			
			6.16			
		LC480	6.25	6.24	0.07	1.14
			6.24			
			6.31			
			6.28			
			6.09			
			6.30			
			6.24			
			6.18			
	Versant kPCR SP	Versant kPCR AD	5.83	5.67	0.14	2.42
			5.65			
			5.42			
			5.89			
			5.61			
			5.64			
			5.60			
			5.71			

Standard deviations in log are calculated between 0.07 and 0.15 log units according to the amplification platforms. These results show an excellent correlation on all the platforms used.

The conversion factor is obtained as follows :

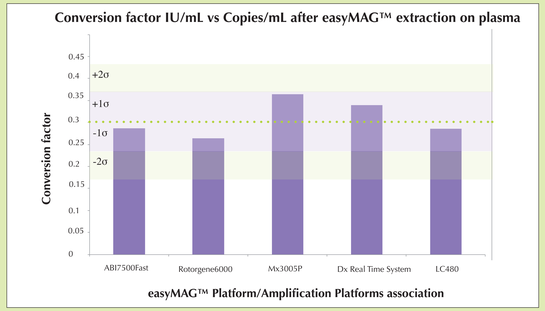
$$\frac{\text{HCMV WHO Quantification (IU/mL)}}{\text{Mean of Argene Quantification (copies/mL)}}$$

Conversion for plasma, easyMAG™ extraction and different 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:



Mean of conversion factor is 0.308 with a standard deviation of 0.04.

Amplification platforms	Copies/mL	Conversion factor	IU/mL	Log IU
ABI 7500Fast	1.75E+04	0.288	5.04E+03	3.70
Rotorgene 6000	2.43E+04	0.265	6.44E+03	3.81
Mx3005P/Versant kPCR AD	1.27E+04	0.365	4.63E+03	3.67
Dx Real Time System	1.42E+04	0.340	4.83E+03	3.68
LC480	1.78E+04	0.286	5.09E+03	3.71

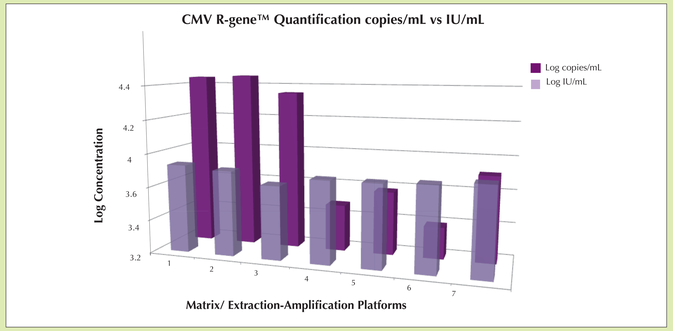
Amplification platforms	Copies/mL	Mean conversion factor	IU/mL	Log IU
ABI 7500Fast	1.75E+04	0.308	5.39E+03	3.73
Rotorgene 6000	2.43E+04		7.48E+03	3.87
Mx3005P/Versant kPCR AD	1.27E+04		3.91E+03	3.59
Dx Real Time System	1.42E+04		4.37E+03	3.64
LC480	1.78E+04		5.48E+03	3.74

Delat Log IU
0.03
0.07
-0.07
-0.04
0.03

Application of conversion factor 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%**	0.8%

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



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

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.

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.