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Collaborative national multicenter for the identification of conversion factors from copies/mL to international units/mL for the normalization of HCMV DNA load

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ABSTRACT

The present multicentric ($n = 11$ laboratories) study aimed to identify conversion factors from copies/mL to international units (IU)/mL for the normalization of HCMV DNA load using the first WHO International Standard for HCMV nucleic acid amplification techniques and to enhance interlaboratory agreement of HCMV DNA quantification methods. Study protocols for whole blood and plasma (extraction and amplification) were performed to calculate conversion factors from HCMV DNA copy number to IU. The greatest variability was observed in samples with lower HCMV concentrations (3.0 Log_{10}) in both biological matrices. Overall, 73.1% (206/282) of whole blood and 82.2% (324/394) of plasma samples analyzed fell within an acceptable variation range (±0.5 Log_{10} difference). An average of 0.64 (range 0.21–1.17) was the conversion factor calculated for the HCMV whole blood panel and 0.82 (range 0.39–2.2) for the HCMV plasma panel.

1. Introduction

Human cytomegalovirus (HCMV) is an important pathogen that causes significant morbidity and mortality in transplant recipients. With more patients undergoing solid organ and hematopoietic stem cell transplantation, along with immunosuppressive drugs and T-cell–depleting regimens, the number of patients at risk for developing HCMV disease is increasing (Boeckh and Ljungman, 2009; Kotton et al., 2010). HCMV viral load testing is routinely used to make decisions regarding diagnosis of active infections, monitoring response to therapy, and when to initiate preemptive therapy (Humar et al., 2002). Quantitative nucleic acid amplification techniques (QNATs) assays, based most commonly on real-time polymerase chain reaction (PCR) techniques, are widely used to measure HCMV replication due to their enhanced sensitivity and specificity, and clinical practice guidelines now recommend their use in preemptive programs for disease prevention (Andrews et al., 2011; Boeckh and Ljungman, 2009; Kotton et al., 2010). One major limitation that has emerged with the use of HCMV PCR testing is the lack of standardization among assays using different calibrators or references (e.g., plasmid-based, genomic, whole virus,

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gene segment) and, consequently, wide variability in output HCMV DNA values usually expressed in copies/mL. In other words, HCMV DNA values obtained from one laboratory could not be necessarily equivalent to a copy reported by another. Additional contributors to assay variability are i) the different HCMV genome regions amplified by commercial or in-house QNATs assays, ii) the use of different biologic specimens (whole blood or plasma), iii) the sample-input volumes, iv) the extraction methods, and v) the elution volumes. Additionally, no consensus on the optimal biological specimens, such as whole blood or plasma, has been achieved (Kotton et al., 2018). In fact, the recently published consensus guideline recommended either whole blood or plasma for QNAT (Kotton et al., 2018). As a result, significant interassay variability complicates the management of transplant patients by hampering the establishment of broadly applicable HCMV DNA cutoff values that can be used in clinical decision making (Boeckh and Ljungman, 2009; Caliendo et al., 2009; Kotton et al., 2010; Pang et al., 2009). Currently, either laboratory-developed HCMV viral load tests or diagnostic processes involving extraction and amplification steps by different commercial companies are validated by individual laboratories with specific and different threshold values for predicting HCMV disease in preemptive therapies. However, this arbitrary validation of methods may impact patient care and limits interinstitutional comparisons.

Recently, the first international standard (IS) for HCMV QNATs was developed and approved by the World Health Organization (WHO) Expert Committee on Biological Standardization (Fryer et al., 2010). This HCMV standard should help to improve interassay agreement among laboratories and enable laboratories to assess the accuracy of viral load values to define threshold values for predicting HCMV disease.

Here, we report the results of a collaborative national multicenter study designed to identify conversion factors from copies/mL to international units (IU)/mL for the normalization of HCMV DNA load using the first WHO International Standard for HCMV nucleic acid amplification techniques to enhance interlaboratory agreement of HCMV DNA quantification. This study involved 11 laboratories participating in the Italian Infections in Transplant Working Group (GLaIT, *Gruppo Lavoro Infezioni nel Trapianto*) in the framework of the Italian Association of Clinical Microbiologists (AMCLI, *Associazione Microbiologi Clinici Italiani*). These study protocols improved agreement in HCMV DNA values among laboratories and the standardization of their reporting in IU.

2. Methods

2.1. GLaIT study protocol

2.1.1. Participating centers and WHO IS for calibration of HCMV QNATs in whole blood and plasma

In 2015, 11 laboratories of the Italian GLaIT started an investigation to calibrate HCMV DNA quantification testing by using the first WHO IS for HCMV. One freeze-dried ampoule of the first WHO International Standard CMV DNA from NIBSC (NIBSC code: 09/1622010) was delivered to participating laboratories with specific instructions for storage and reconstitution. Two different study protocols (specific for whole blood and plasma) were produced by GLaIT to calculate conversion factors (CFs) from copies number/mL to IU/mL.

2.1.2. Preparation of the calibrated reference standard

The WHO IS (NIBSC code 09/162, United Kingdom), consisting of HCMV Merlin strain, previously propagated in MRC-5 cells, was reconstituted with 1 mL of nuclease/free water in order to obtain a defined titer expressed as 5×10^6 HCMV IU/mL.

2.1.3. Sample preparation

The WHO IS was then diluted to nominal concentrations of 5.5, 5.0, 4.5, 4.0, 3.5, and 3.0 Log_{10} IU/mL using whole blood and plasma HCMV-negative (HCMV DNA free specimen) matrices, own supplied in each laboratory. The total volume of each constructed sample was 3.4 mL, sufficient to perform at least three extraction replicates.

2.1.4. Nucleic acid extraction and amplification

Participants were requested to test dilutions using their routine NAT-based assays for HCMV. Three extraction replicates per serial dilution and 2 amplification replicates per extracted sample were prepared including amplification of the HCMV calibrators in triplicate and negative control in duplicate. HCMV calibrators consisted in DNA plasmid were included in the amplification phase and were provided by the manufacturers for commercial amplification kits (in all cases DNA plasmid) or self-produced for laboratories using in-house real-time PCR assays (Table 1).

2.2. Characteristics of the participating laboratories and assays utilized

All 11 GLaIT laboratories participated in the validation procedure for HCMV DNA quantification testing in plasma. Among them, 8 laboratories also participated in validation procedures for whole blood (#1–8; Table 1). The remaining 3 laboratories already used a manufacturer-provided certificated protocol starting from clinical sample extraction to real-time amplification and report of the results. A questionnaire was also sent to obtain technical and methodological information including details on procedures utilized such as extraction instruments, protocols (input/output volumes), extraction kits, real-time PCR instruments, and amplification kits. To ensure confidentiality, all laboratories were requested to send their results and information to the central laboratory in Bologna (Italy) for analysis. Each sample was tested by each of the laboratories using methods adopted for routine virologic monitoring of transplanted patients (Table 1). Nucleic acid extractions were performed using automatic commercially available extractors including QIA Symphony (Qiagen GmbH, Hilden, Germany), NucliSENS EasyMag (bioMérieux, Marcy l'Etoile, France), and MagNAPure 96 (Roche Applied Science, Manheim, Germany) with modifications in input/output volumes. Real-time PCR amplifications were carried out with commercially available kits including HCMV ELITe MGB Kit (ELITech Group S.p.A. Molecular Diagnostics, Italy), HCMV Alert Real-Time (ELITech Group S.p.A. Molecular Diagnostics, Italy), and HCMV r-gene (bioMérieux, Marcy l'Etoile, France) in all of the laboratories with only 2 exceptions, in whom an in-house real-time PCR was performed (Table 1).

2.3. Statistical analysis of panel results

For evaluation of the data sets, the raw values (copies/mL) were first converted to logarithmic (log_{10}) values. The arithmetic mean (AM) and standard deviation (SD) were calculated for all positive samples. Variation between laboratories (interlaboratory) was expressed as the SD of the log_{10} estimates. Log–log linear regression analysis was used for comparison of laboratories' results and the HCMV IS. The Pearson correlation analysis and the Bland–Altman analysis were performed to examine the level of agreement between the laboratories' results and the HCMV IS.

The AM concentration (in Log_{10} copies/mL) for all the WHO IS dilutions was calculated. The CFs for copies/mL to IU/mL were calculated by dividing the stated concentration of the WHO IS dilutions (in IU/mL) by the AM concentration (in copies/ml) reported by each laboratory.

3. Results

A total of 6 concentrations of WHO standard material were assayed in 8 laboratories using whole blood matrix and 11 laboratories using plasma matrix. For each matrix tested (whole blood and plasma), no false-positive results were obtained by any of the GLaIT laboratories (specificity 100%). Regarding sensitivity for the whole blood matrix, dilution #2 (5.0 log_{10} IU/mL) was not detected in 1 of the replicates at 1 center (1/48; 2.0%), while dilution #6 (3.0 log_{10} IU/mL) was not

Table 1
Methods for viral DNA extraction and quantification.

Sample matrix	Center #	Nucleic acid extraction				Amplification						
		Instrument	Protocol	Input volume (μL)	Output volume (μL)	Method	Target gene	Amplicon size	LOD copies/mL	LLQ copies/mL	ULQ copies/mL	Calibrator
Whole blood	1	QIA Symphony	DNA mini kit	200	85	HCMV ELITe MGB Kit, ELITechGroup[a]	UL123	112	151	247	1.4×10^6	DNA Plasmid
	2	QIA Symphony	DNA mini kit	200	115	HCMV ELITe MGB Kit, ELITechGroup[a]	UL123	112	151	247	1.4×10^6	DNA Plasmid
	3	QIA Symphony	DNA mini kit	200	200	HCMV ELITe MGB Kit, ELITechGroup[a]	UL123	112	151	247	1.4×10^6	DNA Plasmid
	4	NucliSENS EasyMag	Generic 2.0.1	100	100	HCMV Alert Real-Time, ELITechGroup[b]	UL123	95	164	316	1.2×10^7	DNA Plasmid
	5	QIA Symphony	DNA mini kit	200	60	HCMV r-gene Argene-Biomerieux[c]	UL83	283	398	500	1.0×10^7	DNA Plasmid
	6	QIA Symphony	Pathogen Midi kit	200	90	HCMV r-gene Argene-Biomerieux[c]	UL83	283	398	500	1.0×10^7	DNA Plasmid
	7	MagNAPure 96	Viral NA small volume kit	200	100	In-house real-time PCR (Mengoli et al., 2004)	UL122	83	1000	NA	NA	DNA Plasmid
	8	QIA Symphony	DNA mini kit	200	90	In-house real-time PCR (Furione et al., 2012)	US8	64	90	90	1.0×10^7	DNA Plasmid
Plasma	1	QIA Symphony	Pathogen Midi kit	400	110	HCMV ELITe MGB Kit, ELITechGroup[a]	UL123	112	293	890	5.0×10^6	DNA Plasmid
	2	QIA Symphony	Pathogen Midi kit	400	90	HCMV ELITe MGB Kit, ELITechGroup[a]	UL123	112	293	890	5.0×10^6	DNA Plasmid
	3	QIA Symphony	Pathogen Midi kit	500	110	HCMV ELITe MGB Kit, ELITechGroup[a]	UL123	112	293	890	5.0×10^6	DNA Plasmid
	4	NucliSENS EasyMag	Generic 2.0.1	400	100	HCMV Alert Real-Time, ELITechGroup[b]	UL123	95	164	316	1.2×10^7	DNA Plasmid
	5	QIA Symphony	Pathogen Midi kit	200	60	HCMV r-gene Argene-Biomerieux[c]	UL83	283	398	500	1.0×10^7	DNA Plasmid
	6	QIA Symphony	Pathogen Midi kit	200	90	HCMV r-gene Argene-Biomerieux[c]	UL83	283	398	500	1.0×10^7	DNA Plasmid
	7	MagNAPure 96	Viral NA small volume kit	200	100	In-house real-time PCR (Mengoli et al., 2004)	UL122	83	1000	NA	NA	DNA Plasmid
	8	QIA Symphony	Pathogen Midi kit	400	90	In-house real-time PCR (Furione et al., 2012)	US8	64	90	90	1.0×10^7	DNA Plasmid
	9	QIA Symphony	Pathogen Midi kit	1000	110	HCMV ELITe MGB Kit, ELITechGroup[a]	UL123	112	293	890	5.0×10^6	DNA Plasmid
	10	NucliSENS EasyMag	Generic 2.0.1	500	100	HCMV ELITe MGB Kit, ELITechGroup[a]	UL123	112	293	890	5.0×10^6	DNA Plasmid
	11	NucliSENS EasyMag	Generic 2.0.1	250	100	HCMV ELITe MGB Kit, ELITechGroup[a]	UL123	112	293	890	5.0×10^6	DNA Plasmid

LOD = lower limit of detection; LLQ = lower limit of quantification; ULQ = upper limit of quantification; NA = not available.
[a] https://www.elitechgroup.com/product/cmv-elite-mgb-open-assays.
[b] https://www.elitechgroup.com/product/q-pcr-alert-kit-microbiology#tab-custom-tab.
[c] https://www.biomerieux-diagnostics.com/sites/clinic/files/9314511-007-gb-a_cmv_r-gene.pdf.

detected in 2 and 3 replicates in 2 centers (5/48; 10.4%) (Table 2). In the plasma panel, a sensitivity of 100% was achieved for all concentrations except 1 (3.0 $\log_{10}$ IU/mL), where 2 replicates of dilution #6 were not detected by 2 centers (2/66; 3.0%).

The overall mean estimates for each dilution point along with the SD (of $\log_{10}$ estimates) for both matrices are summarized in Table 2. The pooled SD for whole blood was 0.30 with a minimum value of 0.25 on dilution #3 (4.5 $\log_{10}$ IU/mL) and a maximum of 0.39 on dilution #6 (3.0 $\log_{10}$ IU/mL). The pooled SD for plasma was 0.37 with a minimum value of 0.30 on dilution #2 (5.0 $\log_{10}$ IU/mL) and a maximum of 0.44 on dilution #4 (4.0 $\log_{10}$ IU/mL). The greatest variability was observed in dilutions with lower amounts of HCMV IS (dilution #6, 3.0 $\log_{10}$ IU/mL) only in whole blood matrix.

A plot of the differences between the $\log_{10}$ IU/mL values for HCMV IS and the results obtained by the GLaIT laboratories was reported using a Bland–Altman analysis. Assuming that difference within ± 0.5 $\log_{10}$ from the "expected results" of the HCMV IS is the acceptable range (Pang et al., 2009), our analysis showed that 73.1% (206/282) and 82.2% (324/394) of the overall results obtained from whole blood and plasma, respectively, fell within this acceptable variation range (Table 3). Regarding the whole blood panel, 76/282 (26.9%) samples had values outside the range of acceptability (values with $>+0.5$ $\log_{10}$ or <-0.5 $\log_{10}$ difference), and in particular, 72/76 (94.9%) had values $>+0.5$ $\log_{10}$ difference, while 4/76 (5.1%) had values <-0.5 $\log_{10}$ difference. All of these out-of-acceptable-range data were distributed over all the standard dilutions analyzed (Table 3). The greatest variation in the quantitative results was observed for samples with the lowest viral load corresponding to sample #6 ($\log_{10}$ 3.0) (Table 3). The 4 (5.1%) samples with values <-0.5 $\log_{10}$ difference were all extracted with MagNA Pure 96™ and amplified with an in-house real-time PCR (Mengoli et al., 2004) (Fig. 1A). All of the 72 samples with values $>+0.5$ $\log_{10}$ difference were extracted using QIAsymphony® and amplified according to different procedures: 53/72 (73.7%) were amplified with HCMV R-gene® (amplificon size 283 bp), 5/72 (6.9%) with the HCMV ELITe MGB Kit (amplificon size 112 bp), and 14/72 (19.4%) with an in-house real-time PCR method (amplificon size 64 bp) (Furione et al., 2012) (Fig. 2A).

In the plasma panel, 70/394 (17.8%) samples had values outside the range of acceptability (values $>+0.5$ $\log_{10}$ or <-0.5 $\log_{10}$ difference); in particular, 47/70 (67.1%) had values $>+0.5$ $\log_{10}$ difference, while 23/70 (32.9%) had values <-0.5 $\log_{10}$ difference (Table 3). These out-of-range signals were distributed over all dilutions of the standard analyzed (Table 3). The greatest variation in the quantitative results was observed for sample #6 ($\log_{10}$ 3.0 IU/mL) with 15/70 (21.4%) out-of-range values. Among samples ($n = 47$) with values $>+0.5$ $\log_{10}$ difference, 35/47 (74.5%) were extracted using QIAsymphony® and 12/47 (25.5%) with EasyMag®. With an analysis of the data according to

Table 2
Summary of performance for WHO HCMV international standard validation in whole blood and plasma matrix.

	Whole blood						Plasma					
	#1 (log 5.5)	#2 (log 5.0)	#3 (log 4.5)	#4 (log 4.0)	#5 (log 3.5)	#6 (log 3.0)	#1 (log 5.5)	#2 (log 5.0)	#3 (log 4.5)	#4 (log 4.0)	#5 (log 3.5)	#6 (log 3.0)
Positivity rate (%)	48/48 (100)	47/48 (97.9)	48/48 (100)	48/48 (100)	48/48 (100)	43/48 (89.6)	66/66 (100)	66/66 (100)	66/66 (100)	66/66 (100)	66/66 (100)	64/66 (96.9)
Mean ± SD (Log_{10} copies/mL)	5.85 ± 0.28	5.31 ± 0.29	4.81 ± 0.25	4.26 ± 0.26	3.76 ± 0.34	3.31 ± 0.39	5.65 ± 0.41	5.19 ± 0.30	4.61 ± 0.36	4.06 ± 0.44	3.58 ± 0.32	2.94 ± 0.41
Overall mean interassay CV (%)	4.82	5.59	5.38	6.28	9.05	12.04	7.35	5.92	7.88	10.90	9.05	14.22
Median (Log_{10} copies/mL)	5.85	5.31	4.82	4.34	3.81	3.37	5.71	5.26	4.73	4.19	3.62	2.94
Range (Log_{10} copies/mL)	5.36–6.4	4.78–5.92	4.44–5.28	3.69–4.66	2.90–4.26	2.24–3.93	4.61–6.70	4.70–6.03	3.4–5.39	2.46–4.70	2.76–4.06	1.57–3.71

SD = standard deviation; CV = coefficient of variation.

amplification procedure, it was shown that 35/47 (74.5%) were amplified with HCMV R-gene® (amplicon size 283 bp), 8/47 (17.0%) with the HCMV ELITe MGB Kit (amplicon size 112 bp), and 4/47 (8.5%) with the HCMV Alert Real-Time kit (amplicon size 95 bp). Among samples ($n = 23$) with values <−0.5 log_{10} difference, 16/23 (69.6%) were extracted using QIAsymphony® and 7/23 (30.4%) with MagNA Pure 96™. Of these, 15/23 (65.3%) were amplified with the HCMV ELITe MGB Kit, 7/23 (30.4%) with an in-house assay (Mengoli et al., 2004), and 1/23 (4.3%) with HCMV R-gene®.

In order to evaluate the source of variability, the log_{10} differences between the GLaIT laboratory results and the IS values were compared according to the 2 most common extraction methods (EasyMag® vs QIAsymphony®) and real-time PCR amplification kits (HCMV R-gene® vs ELITe MGB®). In the HCMV whole blood panel, the median log_{10} difference was higher for QIAsymphony® (0.41, range −0.30 to +0.93) as compared with EasyMag® (0.01, range −0.33 to +0.34; $P < 0.001$) (Fig. 1A) and for HCMV R-gene® (0.63, range −0.30 to +0.92) as compared with ELITe MGB® (0.32, range −0.22 to +0.55; $P < 0.001$)(Fig. 2A). In the HCMV plasma panel, the median log_{10} difference was higher for EasyMag® (0.37, range −0.21 to +0.59) as compared with QIAsymphony® (−0.03, range −1.43 to +1.20; $P < 0.001$) (Fig. 1B) and for HCMV R-gene® (0.49, range −0.94 to +1.20) as compared with ELITe MGB® (−0.04, range −1.43 to +0.58; $P < 0.001$) (Fig. 2B).

Finally, CFs (for copies/mL to IU/mL) were calculated by dividing the stated concentration of the each dilution by the median concentration (in copies/mL) and were determined for each GLaIT laboratory and matrix (Table 4). In detail, a mean of 0.63 (range 0.21–1.17) was the CF calculated for the HCMV whole blood panel and 0.82 (range 0.39–2.2) for the HCMV plasma panel.

4. Discussion

The mission of the GLaIT group is to improve standardization of diagnostic procedures in order to improve uniformity of HCMV PCR results used in the monitoring of solid organ and stem cell transplant recipients in Italy. Here, we describe a national multicenter study for the identification of conversion factors from copies/mL to IU/mL for the normalization of HCMV DNA load using the first WHO International Standard for HCMV nucleic acid amplification techniques to improve interlaboratory agreement in HCMV DNA quantification. The availability of viral standards should facilitate clinical trials in the future and also enhance the performance of clinical laboratories in patient care.

The validation procedures on whole blood and plasma panels, described in our study, were developed by the GLaIT laboratories, where 6-fold serial dilutions of HCMV IS were tested by using routine NAT-based assays for HCMV. No false-positive results were obtained by any of the GLaIT laboratories, reaching a specificity of 100%. Regarding sensitivity, as expected, the greatest variability was observed in samples with lower HCMV concentrations (3.0 log_{10} in both panels).

It has been suggested that quantitative HCMV DNA results from individual samples should not differ by more than ±0.5 log_{10} IU/mL (Kraft et al., 2012). Our study was designed to evaluate result variability according to extraction and amplification methods in both whole blood and plasma samples. In contrast with previous studies in which variability was sometimes >4.0 log_{10} copies/mL and seldom <2.0 log_{10} copies/mL (Hayden et al., 2013; Hirsch et al., 2013; Pang et al., 2009), in our study, only 26.6% of whole blood and 18.3% of plasma samples yielded results outside the range of acceptability (values with >+0.5 log_{10} or <−0.5 log_{10} difference). As previously observed, the variability in whole blood matrix was greater in comparison with the plasma panel (Fryer et al., 2016a, b; Jones et al., 2016). Indeed, viral load changes within ±0.5 log_{10} IU/mL are considered nonsignificant in the management of an individual patient, and the matrix effect observed in our study has the potential to introduce a systematic bias between laboratories that standardize relative to the WHO IS by using whole blood versus

Table 3
Summary of serial dilutions (from 3.0 to 5.5 Log_{10} HCMV IU/mL) outside and within the range of acceptability (± 0.5 Log_{10}).

Log_{10} international HCMV standard dilution	Whole blood No. (%)			Plasma No. (%)		
	<−0.5	>−0.5 and <0.5[a]	>0.5	<−0.5	>−0.5 and <0.5[a]	>0.5
3	3 (7.0)	26 (60.5)	14 (32.5)	9 (14.1)	49 (76.6)	6 (9.3)
3.5	1 (2.1)	35 (72.9)	12 (25.0)	3 (4.5)	56 (84.8)	7 (10.6)
4	-	38 (79.2)	10 (20.8)	6 (9.1)	52 (78.8)	8 (12.1)
4.5	-	35 (72.9)	13 (27.1)	3 (4.5)	57 (86.4)	6 (9.1)
5	-	37 (78.7)	10 (21.3)	-	57 (86.4)	9 (13.6)
5.5	-	35 (72.9)	13 (27.1)	2 (3.0)	53 (80.3)	11 (16.7)
Total	4 (1.5)	206 (73.1)	72 (25.4)	23 (5.8)	324 (82.2)	47 (12.0)

[a] Acceptable Δ Log range.

plasma as the matrix. In HCMV whole blood panels, the greatest variation was observed for samples with the lowest HCMV viral load extracted using QIAsymphony® and amplified using HCMV R-gene® and some in-house assays (Furione et al., 2012; Mengoli et al., 2004). It was recently reported in 2 studies including at least 8 different amplification assays that small amplicons resulted in high viral loads as compared to those yielded by larger amplicons (Hayden et al., 2015; Preiksaitis et al., 2016). In our study, only 5 amplification assays were analyzed, and the great majority of results with viral load >+0.5 log_{10} difference were obtained by assay with larger amplicon size (283 bp, HCMV R-gene®). Therefore, in our study, the amplicon size "effect" seems to be less determinant in increasing variability as compared to previous studies (Hayden et al., 2015; Preiksaitis et al., 2016).

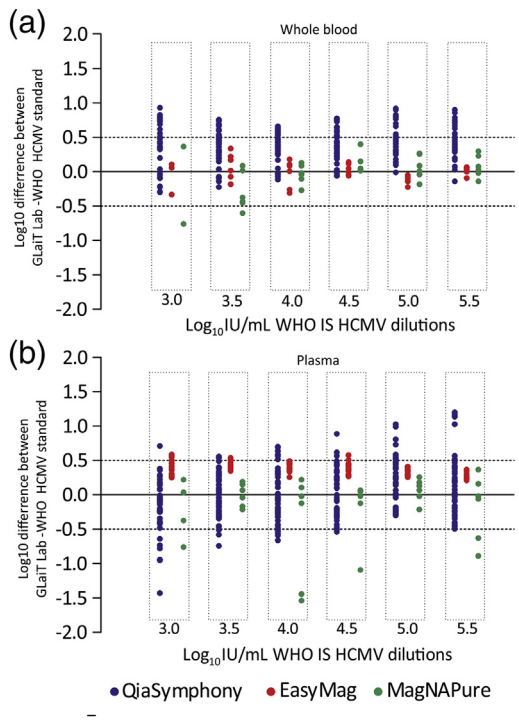

Fig. 1. Bland–Altman plots. Log_{10} variation in reported results for individual positive samples, according to extraction methods for (A) whole blood and (B) plasma, relative to "expected values" (assigned 0 value) corresponding to 6 half-log serial dilution (5.5, 5.0, 4.5, 4.0, 3.5, and 3.0 Log_{10}). Dotted lines indicate 0.5 log_{10} above and below the "expected value."

The main objective of the present study was to define conversion factors to copies/mL to IU/mL to fully calibrate the HCMV QNATs assays. In this perspective, reporting data using both copies/mL and IU/mL for a limited time period may be useful to help clinicians that will have to get used to new units. For sure, as previously occurred for hepatitis C, time has come for HCMV to report quantitative results using IU/mL (Caliendo, 2013). In our study, the WHO IS was used to calculate a HCMV CF to IU/mL for each GLaIT laboratory ($n = 11$ centers) for both whole blood and plasma matrices. The average of CF was 0.63 (SD 0.36) for whole blood and 0.82 (SD 0.59) for plasma. In agreement with others, the results of the present study suggest that using IS as a common assay calibrator also improves result harmonization for clinical samples (Kraft et al., 2012; Pang et al., 2009). This study has allowed all GLaIT laboratories to recalibrate their assays to finally report HCMV quantitative results only in IU/mL.

Based on our results, the matrix effect on HCMV quantification seems to be within acceptable limits (SD <0.50 log_{10}), but further studies are needed to achieve consistent standardization across different detection methods (Fryer et al., 2016a, b). The interlaboratory result variability observed in our multicenter study together with the CFs calculated for the standardization of viral load in IU/mL might help with transplant recipient management and the determination of clinically relevant widely accepted quantitative thresholds for initiating antiviral therapy. However, a further validation on clinical samples should be performed to validate the CFs obtained in the present study.

In conclusion, an HCMV CF to IU/mL was calculated by each GLaIT laboratory for different matrices. The development and the standardization of quantitative HCMV DNA testing in whole blood and plasma, reporting HCMV load using IU/mL, should significantly improve agreement in viral load values between laboratories. However, the study here presented represents an initial step in making laboratories familiar with the concept of reporting HCMV NAT in IU/mL. Therefore, a next-step study involving GLaIT group laboratories with blind HMCV-positive samples confirming the validity of CFs obtained here should be performed. Finally, the standardization of HCMV results may allow the establishment of a broadly applicable cutoff (in IU/mL) for initiating preemptive therapy that can be used in clinical decision making.

Conflict of interest information

The authors have no conflicts of interest to declare.

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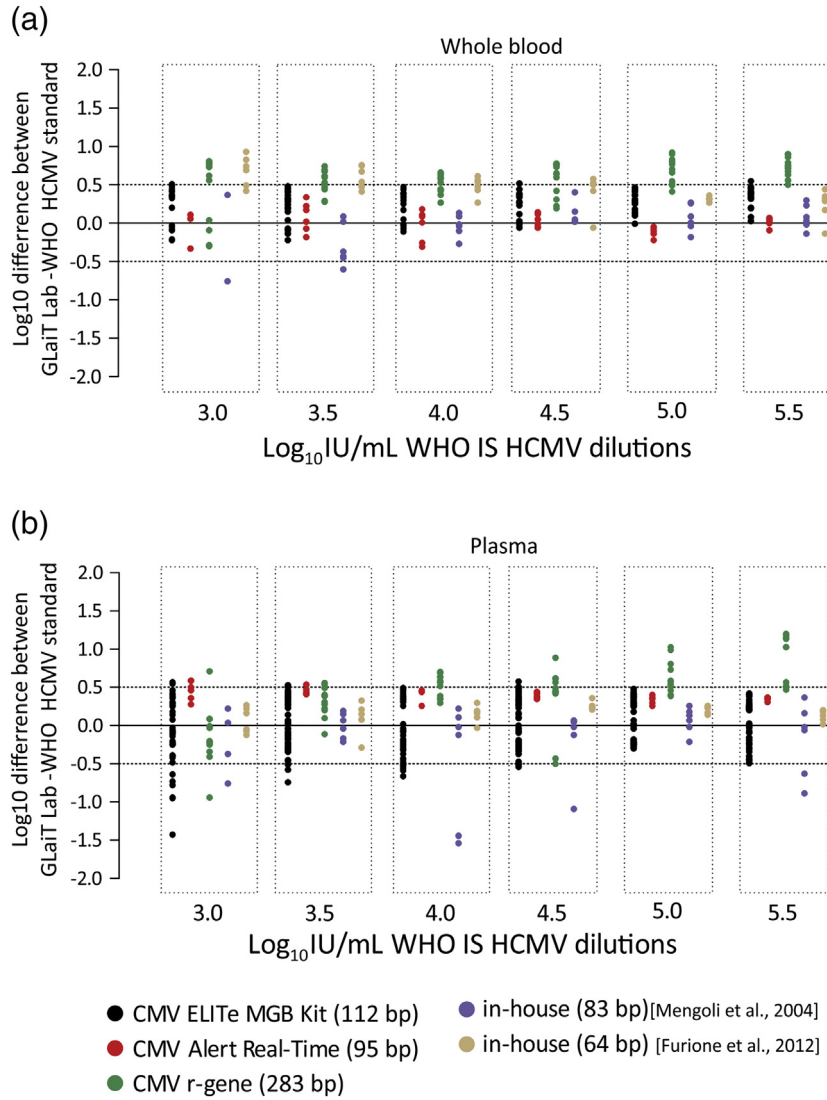

Fig. 2. Bland–Altman Plots. Log_{10} variation in reported results for individual positive samples, according to amplification kit for (A) whole blood and (B) plasma, relative to "expected values" (assigned 0 value) corresponding to 6 half-log serial dilution (5.5, 5.0, 4.5, 4.0, 3.5, and 3.0 Log_{10}). Dotted lines indicate 0.5 log_{10} above and below the "expected value." Amplicon size (bp) of the gene targeted is reported for each HCMV DNA quantification assay.

provided first WHO International Standard CMV DNA from NIBSC (NIBSC code: 09/162 2010) free of charge. We thank Daniela Sartori for careful preparation of the manuscript and Laurene Kelly for English revision.

Table 4
Summary of HCMV conversion factor to IU/mL reported by 11 GLaIT laboratories.

GLaIT laboratory	Conversion factor copies/mL → IU/mL	
	Whole blood	Plasma
1	0.92	2.2
2	0.46	0.98
3	0.42	0.47
4	1.08	0.39
5	0.21	0.42
6	0.43	0.42
7	1.17	1.03
8	0.40	0.69
9		1.57
10		0.47
11		0.39
Mean ± SD	0.63 ± 0.36	0.82 ± 0.59

References

Andrews PA, Emery VC, Newstead C. Summary of the British Transplantation Society guidelines for the prevention and management of CMV disease after solid organ transplantation. Transplantation 2011;92:1181–7. https://doi.org/10.1097/TP.0b013e318235c7fc.

Boeckh M, Ljungman P. How we treat cytomegalovirus in hematopoietic cell transplant recipients. Blood 2009;113:5711–9. https://doi.org/10.1182/blood-2008-10-143560.

Caliendo AM. The long road toward standardization of viral load testing for cytomegalovirus. Clin Infect Dis 2013;56:374–5. https://doi.org/10.1093/cid/cis905.

Caliendo AM, Shahbazian MD, Schaper C, Ingersoll J, Abdul-Ali D, Boonyaratanakornkit J, et al. A commutable cytomegalovirus calibrator is required to improve the agreement of viral load values between laboratories. Clin Chem 2009;55:1701–10. https://doi.org/10.1373/clinchem.2009.124743.

Fryer JF, Heath AB, Anderson R, Minor PD and the Collaborative Study Group. Collaborative study to evaluate the proposed 1st WHO international standard for human cytomegalovirus (HCMV) for nucleic acid amplification (NAT)–based assays. 2010; WHO/BS/10.2138:1–40. http://www.who.int/iris/handle/10665/70521.

Fryer JF, Heath AB, Minor PD, Collaborative Study Group. a collaborative study to establish the 1st WHO international standard for human cytomegalovirus for nucleic acid amplification technology. Biologicals 2016a;44:242–51. https://doi.org/10.1016/j.biologicals.2016.04.005.

Fryer JF, Morris CL, Almond NM, Minor PF. Commutability of the World Health Organization international standard for human cytomegalovirus: standard or assay. J Clin Microbiol 2016b;54:3073. https://doi.org/10.1128/JCM.01706-16.

Furione M, Rognoni V, Cabano E, Baldanti F. Kinetics of human cytomegalovirus (HCMV) DNAemia in transplanted patients expressed in international units as determined

with the Abbott RealTime CMV assay and an in-house assay. J Clin Virol 2012;55:317–22. https://doi.org/10.1016/j.jcv.2012.08.017.

Hayden RT, Preiksaitis J, Tong Y, Pang X, Sun Y, Tang L, et al. Commutability of the first World Health Organization international standard for human cytomegalovirus. J Clin Microbiol 2015;53:3325–33. https://doi.org/10.1128/JCM.01495-15.

Hayden RT, Shahbazian MD, Valsamakis A. J. Boonyaratanakornkit J, Cook L, Pang XL et al. Multicenter evaluation of a commercial cytomegalovirus quantitative standard: effects of commutability on interlaboratory concordance. J Clin Microbiol 2013;51:3811–7. https://doi.org/10.1128/JCM.02036-13.

Hirsch HH, Lautenschlager I, Pinsky BA, Cardeñoso L, Aslam S, Cobb B, et al. An international multicenter performance analysis of cytomegalovirus load tests. Clin Infect Dis 2013;56:367–73. https://doi.org/10.1093/cid/cis900.

Humar A, Kumar D, Boivin G, Caliendo AM. Cytomegalovirus (CMV) virus load kinetics to predict recurrent disease in solid-organ transplant patients with CMV disease. J Infect Dis 2002;186:829–33. https://doi.org/10.1086/342601.

Jones S, Webb EM, Barry CP, Choi WS, Abravaya KB, Schneider GJ, et al. Commutability of cytomegalovirus WHO international standard in different matrices. J Clin Microbiol 2016;54:1512–9. https://doi.org/10.1128/JCM.03292-15.

Kotton CN, Kumar D, Caliendo AM, Asberg A, Chou S, Snydman DR, et al. International consensus guidelines on the management of cytomegalovirus in solid organ transplantation. Transplantation 2010;89:779–95. https://doi.org/10.1097/TP.0b013e3181cee42f.

Kotton CN, Kumar D, Caliendo AM, Huprikar S, Chou S, Danziger-Isakov L et al. The Transplantation Society International CMV Consensus Group. The third international consensus guidelines on the management of cytomegalovirus in solid-organ transplantation. Transplantation 2018;102:900–31. https://doi.org/10.1097/TP.0000000000002191.

Kraft CS, Armstrong WS, Caliendo AM. Interpreting quantitative cytomegalovirus DNA testing: understanding the laboratory perspective. Clin Infect Dis 2012;54:1793–7. https://doi.org/10.1093/cid/cis212.

Mengoli C, Cusinato R, Biasolo MA, Cesaro S, Parolin C, Palù G. Assessment of CMV load in solid organ transplant recipients by pp65 antigenemia and real-time quantitative DNA PCR assay: correlation with pp67 RNA detection. J Med Virol 2004;74:78–84. https://doi.org/10.1002/jmv.20149.

Pang XL, Fox JD, Fenton JM, Miller GG, Caliendo AM, Preiksaitis JK. American Society of Transplantation Infectious Diseases Community of Practice, Canadian Society of Transplantation. Interlaboratory comparison of cytomegalovirus viral load assays. Am J Transplant 2009;9:258–68. https://doi.org/10.1111/j.1600-6143.2008.02513.x.

Preiksaitis JK, Hayden RT, Tong Y, Pang XL, Fryer JF, Heath AB, et al. Are we there yet? Impact of the first international standard for cytomegalovirus DNA on the harmonization of results reported on plasma samples. Clin Infect Dis 2016;63:583–9. https://doi.org/10.1093/cid/ciw370.