Fully automated quantification of cytomegalovirus (CMV) in whole blood with the new sensitive Abbott RealTime CMV assay in the era of the CMV international standard.

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ABSTRACT

Fully standardized reproducible and sensitive quantification assays for cytomegalovirus (CMV) are needed to better define thresholds for antiviral therapy initiation and interruption. We evaluated the new released Abbott RealTime CMV assay for CMV quantification on whole blood (WB) that includes automated extraction and amplification (m2000 RealTime system). Sensitivity, accuracy, linearity and intra- and inter-assay variability were validated in WB matrix using quality control panels (QCMD) and the WHO international standard (IS). Intra- and inter-assay coefficients of variation were 1.37% and 2.09% at 5 log10 copies/ml and 2.41% and 3.80% at 3 log10 copies/ml, respectively. According to QCMD and Abbott RealTime CMV expected values, the lower limit of quantification was 104 and below 50 copies/ml, respectively. The conversion factor between international units and copies (2.18), determined from serial dilutions of the WHO IS in WB, was significantly different from the factor provided by the manufacturer (1.56) (P = 0.001). Results from 302 clinical samples were compared to the Qiagen/artus CMV assay on the same m2000 RealTime system. The two assays provided highly concordant results (concordance correlation coefficient = 0.92), but Abbott RealTime CMV assay detected and quantified respectively 20.6% and 47.8% more samples than Qiagen/artus CMV assay. The sensitivity and reproducibility results along with automation fulfill quality requirements to implement Abbott RealTime CMV assay in clinical settings. Our results stress out the need for careful validation of conversion factors of the WHO IS in WB provided by manufacturers to allow future comparison of results obtained with different assays.
INTRODUCTION

Cytomegalovirus (CMV), a member of the *Herpesviridae* family, establishes a life-long persistent and latent infection within its host. Reactivations occur frequently throughout the life with asymptomatic viral shedding in healthy individuals. However in immunocompromised patients, especially in allogeneic hematopoietic stem cell or solid-organ transplantation recipients, CMV may cause severe disease (1, 2). Viremia is a well-recognized risk factor for CMV disease and viral loads kinetics has been reported to be predictive for the development of CMV disease (3, 4). Quantitative viral genome testing in blood (whole blood or plasma) is the best option for diagnosis, decision making regarding initiation of preemptive therapy and monitoring response to therapy (5, 6). The reliability and accuracy in the viral load determination are then critical points for the management of transplant patients. Due to better sensitivities and reduced technical hand time, quantitative real-time PCR assays for quantification of CMV DNA have largely replaced the pp65-antigenemia testing in routine diagnostic laboratories (7, 8).

Because of the increased needs in quality certification requirements of laboratories, there is a trend for the use of IVD/CE labeled or FDA-approved commercial assays. However, commercially available assays may differ significantly between each other for the measurement of CMV DNA loads in particular according to the extraction method used which is a source of variability with whole blood (WB) (9-11). An interlaboratory comparison of CMV viral load assays in 33 different laboratories over USA, Canada and Europe has shown a significant variability with more than of 40% results with a delta log above 0.5, which may impact patient care and limit inter-institutional comparisons (12). Two other studies conducted in USA have reported interlaboratory comparison of CMV viral load measures with both in-house and commercial assays and have shown significant differences in quantitative values ranging to 2 up to 3 log_{10} between two laboratories (13, 14). In order to
standardize results and overcome the variability between laboratories, the World Health Organization (WHO) Expert Committee on Biological Standardization has established the first International Standard (IS) for human cytomegalovirus for Nucleic Acid Amplification Techniques by November 30th 2010 (15). For each assay, a conversion factor is applied in order to provide the results in International Units per ml (IU/ml).

In this study we report the evaluation of the recently available IVD/CE-labeled RealTime CMV assay (Abbott RealTime CMV) that includes a complete fully automated extraction and amplification of CMV DNA from WB on the m2000 RealTime system. The analytical performances have been validated in WB matrix using quality control panels and the IS. Clinical samples were tested and the results compared to those obtained with the Qiagen/Artus IVD/CE-labeled CMV assay on the m2000 RealTime system. Our results demonstrate a good reliability and accuracy of results.

MATERIALS AND METHODS

Standards and clinical samples. (i) QCMD samples. The QCMD 2010 (Quality Control for Molecular Diagnostics) CMV proficiency panel (QCMD, Scotland) consisted of 10 lyophilized samples (QCMD CMV 10-01 to QCMD CMV 10-10). Nine samples contained various concentrations of cultured CMV strain AD169 in either virus transport medium or human plasma and one plasma sample was negative for CMV. In positive samples, CMV DNA concentrations ranged from 230 to 2,552,701 copies/ml (i.e. 2.36 to 6.41 log_{10} copies/ml). (ii) WHO International Standard (WHO IS). The WHO IS for human CMV for Nucleic Acid Amplification Techniques (National Institute for Biological Standards and Control [NIBSC] code: 09/162, NIBSC, Hertfordshire, Great Britain) is a lyophilized whole virus preparation of the CMV Merlin strain (15). After reconstitution in 1 ml of water, the WHO IS has a concentration of 5x10^6 IU/ml (i.e. 6.7 log_{10} IU/ml). (iii)
Clinical samples. A total of 302 blood specimens received in the laboratory for CMV load quantification from November 2010 to December 2010 were selected if at least two aliquots of 0.5 ml of WB collected in EDTA tubes were stored at -80°C. They were collected from 207 patients, including 14 hematopoietic stem cell transplant (HSCT) recipients, 94 kidney transplant recipients, 47 HIV-infected patients, 27 patients with leukemia or lymphoma and 11 patients with inflammatory bowel disease. The clinical samples were tested in singlet with the Abbott RealTime CMV assay and with the test of record Qiagen/Artus CMV assay in separate runs.

Quantitative real-time PCR assays. (i) Abbott RealTime CMV assay. Quantification of CMV in WB was carried out with the Abbott RealTime CMV (Abbott Molecular Inc., Des Plaines, Il, USA) on the m2000 RealTime platform that includes the m2000sp instrument for automated extraction of DNA and the m2000rt instrument for the real time PCR in batches of 48 tests. Extraction of DNA was done with the Sample Preparation System DNA Kit. The Abbott RealTime CMV assay uses three reagents kits: Amplification Reagent Kit for the amplification, Control Kit for external controls and Calibrator Kit for the standard curve. The amplification targets two highly conserved regions, within the UL34 and UL80.5 genes, respectively. An internal control (IC) is also supplied to check the overall process including DNA extraction and possible PCR inhibition. Automated DNA extraction was performed from 500 µl WB (processed 300 µl, eluted in 150 µl) in the Abbott m2000sp instrument, followed by automated addition into the PCR plate of the master mixture and DNA extracts (volume used for PCR: 20 µl, corresponding to 40 µl of WB). The sealed PCR plate was loaded on the m2000rt instrument for quantification of viral CMV DNA. Two controls (one positive and one negative) provided by the manufacturer are included in each run. Two calibrators (A and B) analyzed in triplicate were used to establish the standard curve and calculated the CMV DNA concentration in samples. The results are expressed in copies/ml or...
log_{10} copies/ml, as follows: no detection of amplification signal = “Not Detected”, detection of amplification signal with value under 1.60 log_{10} copies/ml = “Detected”, absolute values for quantification between 1.60 \text{ to } 8.00 \log_{10} \text{ copies/ml}, and for values above 8.00 log_{10} copies/ml = “Upper limit of quantification”. (ii) Qiagen/Artus CMV assay. Real-time PCR with the CMV ABI Prism SDS Kit (Qiagen Hamburg GmbH, Hilde, Germany) was also performed on the m2000 RealTime platform in batches of 48 tests. The Qiagen/Artus CMV kit contains reagents and enzymes for the specific amplification of a 105 bp region of the CMV major immediate early gene. DNA extractions were performed using the Abbott m2000 Sample Preparation System DNA kit. CMV DNA was extracted from 500 µl of WB (processed 300 µl) including IC spiking and eluted in a final volume of 150 µl. According to the manufacturer’s instructions, PCR was carried out in a 96-well plate with a reaction volume of 50 µl containing 20 µl of DNA extract and 30 µl of Master mixture. Four external positive controls (CMV QS 1–4) were used to establish the standard curve. Controls are DNA plasmids containing either the CMV target sequence (QS) or a heterologous target sequence (IC). The results are given in copies/ml or log_{10} copies/ml, as follows: no detection of amplification signal = “Not Detected”, detection of amplification signal with value under 2.30 log_{10} copies/ml = “Detected”, absolute values for quantification between 2.30 \text{ to } 8.00 \log_{10} \text{ copies/ml}, and for values above 8.00 log_{10} copies/ml = “Upper limit of quantification”.

Analytical performances of the Abbott RealTime CMV assay. (i) The lower limit of quantification (LLQ) was determined first by using serial dilutions of the QCMD CMV 10-08 sample (23988 copies/ml; i.e. 4.38 log_{10} copies/ml) at expected values of 200, 150, 100 and 50 copies/ml. The first dilution was prepared by adding 417 µl of QCMD CMV 10-08 sample to 50 ml of CMV DNA negative WB. The LLQ was determined secondarily by using serial dilutions of clinical samples with expected viral loads of 150, 100 and 50 copies/ml with the Abbott RealTime CMV assay. Serial dilutions were generated starting with a fifty
fold dilution of a pool of 6 WB positive samples at 4 log\textsubscript{10} copies/ml in CMV negative WB.

For the LLQ based on QCMD and Abbott RealTime CMV expected values, each dilution was tested 28 to 30 times. (ii) The between-run and within-run reproducibility was determined at 3 log\textsubscript{10} copies/ml and 5 log\textsubscript{10} copies/ml by diluting serially in CMV DNA negative WB the remainder of 6 CMV DNA positive WB samples with values around 6 log\textsubscript{10} copies/ml. Thirty and eleven replicates were tested for intra-assay and inter-assay reproducibility, respectively.

(iii) The linearity of the assay was verified with dilutions of a highly CMV DNA positive sample in CMV negative WB. These dilutions had expected viral loads ranging from 1.7 to 5 log\textsubscript{10} copies/ml. For each dilution, CMV DNA was quantified with the Abbott RealTime CMV assay at least three times and the mean concentration was calculated.

**Concordance correlation coefficient.** To assess the agreement of the two methods, we use the concordance correlation coefficient (CCC) (16). A value of 1 denotes a perfect concordance; a value of zero denotes its complete absence. In our work, as the data were left-censored by the minimal detected value of each method, we use the maximum likelihood estimator proposed by Barnhart et al. to estimate the CCC (17). 95% confidence intervals (95% CI) are given and were assessed using bootstrap.

**Conversion factor between copies/ml and IU/ml.** The conversion factor between copies/ml and IU/ml was evaluated with the Abbott RealTime assay. Serial dilutions of the WHO IS were prepared in PBS (PBS\_dilutions) and in WB (WB\_dilutions) with expected values of 5, 4 and 3 log\textsubscript{10} IU/ml. In detail, 200 µl of the reconstituted WHO IS was added to 9.8 ml of PBS (1:50 dilution) to constitute the PBS\_dilution\#1 at an expected concentration of 5 log\textsubscript{10} IU/ml and the mixture was homogenized on a rotary shaker for 20 minutes. Two successive ten-fold dilutions were carried out to obtain PBS\_dilution\#2 (4 log\textsubscript{10} IU/ml) and PBS\_dilution\#3 (3 log\textsubscript{10} IU/ml). Each dilution was split in aliquots of 600 µl. WB\_dilution\#1, WB\_dilution\#2 and WB\_dilution\#3 were prepared following the same protocol. To assess the
conversion factor in PBS and WB, 11 replicates of the three PBS_dilutions and the three
WB_dilutions were analyzed in the same run.

Statistical analysis. Analyses were performed using the statistical R package (2.15.0)
(http://www.R-project.org/). A \( P \) value < 0.05 was considered to be a statistical significance.

(i) Determination of the LLQ. Parameters of the detection-number of copies curve were
estimated using a probit regression model. The LLQ defined as the 95th percentile of the
previous model was then estimated. (ii) Linearity. Observed values were plotted against
theoretical values. Parameters of the corresponding linear regression model were estimated.

(iii) Conversion factor between copies/ml and IU/ml. Conversion factors for the number of
copies were estimated using a linear regression model (observed values against theoretical
values) without intercept. Conversion factors for log\(_{10}\) of copies were estimated using the
mean difference between the observed and the theoretical value. 95% CI are given and tests
against values given by the Abbott RealTime CMV assay were performed.

RESULTS

Analytical performances of the Abbott RealTime CMV assay. The LLQ, between-run and
within-run reproducibility and linearity of the assay were determined by using dilutions of
QCMD or clinical samples positive for CMV DNA. As the assay is CE marked for whole
blood, the dilutions were performed in CMV negative WB. (i) Lower limit of quantification.
The LLQ for WB was defined as the CMV viral load detected by the assay with the
probability of 95% and tested with dilutions of QCMD samples and clinical samples. Probit
analysis of the data predicted a LLQ at 104 copies/ml (95% CI: 81-122 copies/ml) with the
QCMD dilutions (Table 1). All dilutions of clinical samples with expected values of 150 and
100 copies/ml and 96.7% with expected values of 50 copies/ml were detected. (ii) Between-
run and within-run reproducibility. Intra-assay coefficients of variation (CV), determined
on 30 replicates, were 1.37% and 2.41% at the mean values of 5.09 and 3.04 log_{10} copies/ml, respectively. The same two samples were tested in 11 different assays. Testing showed CV of 2.09% and 3.80% for 5.01 and 2.95 log_{10} copies/ml, respectively. The variability of the detection of the IC supplied with Abbott RealTime CMV assay to check the overall process including DNA extraction and possible PCR inhibition was evaluated on the clinical samples. Overall through the 302 samples tested in 14 separate experiments, no inhibition was detected and the mean cycle threshold (Ct) value (±SD) was 29.28 (± 0.30). The intra-run variation coefficients ranged from 0.01% to 1.71%. The inter-run variation coefficient calculated with the means of Ct values of each run was 0.43%. (iii) **Linearity.** The assay was linear (r²=0.9682) in the range of all samples tested (1.7 to 5 log_{10} copies/ml) (not shown).

**Correlation with QCMD values.** CMV DNA was not detectable in the negative QCMD CMV 10-05 sample and was quantified for the other 9 positive samples (Table 2). CMV DNA loads measured by the Abbott RealTime CMV assay were lower than those expected. Differences between measured and expected values ranged from -0.13 (QCMD CMV 10-02) to -0.55 log_{10} copies/ml (QCMD CMV 10-08), with a mean of -0.26 log_{10} copies/ml. When viral loads were expressed in copies/ml, the conversion factor between measured and expected values was 1.81 (95% CI: 1.78 to 1.83).

**Comparison of the Abbott RealTime CMV and Qiagen/Artus CMV assays.** The 302 clinical samples were analyzed using both analytical systems. By using the Abbott RealTime CMV assay, CMV DNA was detected in 129 (42.7%) clinical samples. For 99 of them (32.8%), DNA was quantifiable (above 1.60 log_{10} copies/ml). With the Qiagen/Artus CMV assay, CMV DNA was detected in 107 (35.4%) samples of which 67 (22.2%) were quantified above the LLQ value (2.30 log_{10} copies/ml). Compared to Qiagen/Artus CMV assay, the Abbott RealTime CMV assay provided thus an increase of 20.6% and 47.8% of samples detected and quantified, respectively.
Results between the two techniques were discordant for 38 (12.6%) samples. Discrepancies were observed only for samples with low copies numbers, mostly below the lower quantification limit. In 8 of them, CMV DNA was undetectable by using the Abbott RealTime CMV assay but detected by using the Qiagen/Artus CMV assay (DNA load below the LLQ for 6 samples and at 2.36 and 2.78 log_{10} copies/ml for the two other samples, respectively).

Conversely, CMV DNA was undetectable with the Qiagen/Artus CMV assay in 30 samples, but detected in 23 of them (DNA load below the LLQ) or quantified in the 7 other samples (median DNA load: 2.00 log_{10} copies/ml; range, 1.67 to 2.18) with the Abbott RealTime CMV assay. To further analyze the 30 Abbott RealTime CMV positive and Qiagen/Artus CMV negative samples, we examined if previous or subsequent samples collected within two weeks in the same patients were tested with the Qiagen/Artus CMV assay. For the 30 discrepant samples, 15 previous or subsequent samples were available (including 13 tested in this study) and all were positive.

The two assays showed for the 65 positive samples quantified with both assays a good concordance with a CCC of 0.92 (95% CI: 0.62 to 0.98). Viral load values measured with the Qiagen/Artus CMV assay were on average 0.23 log_{10} higher than those measured by the Abbott RealTime CMV assay (P = 0.0003). Bland Altman analysis on the same 65 samples showed that 41 (63.1%) and 63 (96.9%) of them had a variation below 0.5 log_{10} and 1.0 log_{10} copies/ml, respectively (Fig. 1).

In order to further analyze the correlation between the two assays, we compared the viral load kinetics for patients with at least three successively positive samples. As shown in Fig. 2, the profiles are very similar with overlapping patterns in all patients. In addition, variations were always in the same direction except between the second and third sample for patient #72 (decrease of 0.08 log_{10} copies/ml with the Qiagen/Artus CMV assay and increase of 0.15 log_{10} with the Abbott RealTime CMV assay) and the first and second sample for patient #182.
Determination of conversion factor between international units and copies. Boxplots of conversion factors calculated for the 11 replicates of each dilution of the WHO IS in PBS (PBS_dilution#1, PBS_dilution#2 and PBS_dilution#3) or in WB (WB_dilution#1, WB_dilution#2 and WB_dilution#3) are depicted in Fig. 3. In PBS, the conversion factor varied according to the dilution tested (Fig. 3A). In WB, the conversion factor was roughly constant and always higher than the manufacturer's value (Fig. 3B). The seemingly wide dispersion of conversion factor values for the WB_dilution#1 was due only to one extreme value (8.04) which was however not rejected.

The conversion factors determined using linear regression models were in copies/ml 1.16 (95% CI: 1.11 to 1.22, test against Abbott RealTime value [1.56]: $P < 0.0001$) for PBS and 2.18 (95% CI: 1.79 to 2.80, test against Abbott RealTime value [1.56]: $P = 0.001$) for WB and in log10 copies/ml 0.15 (95% CI: 0.11 to 0.18, test against Abbott RealTime value [0.19]: $P = 0.02$) for PBS and 0.45 (95% CI: 0.38 to 0.52, test against Abbott RealTime value [0.19]: $P < 0.0001$) for WB. The calculated conversion factor in WB was thus significantly different compared with the manufacturer’s conversion factor.

DISCUSSION

Sensitive and reproducible quantification of CMV DNA is crucial for the initiation and the monitoring of therapy to control CMV infection in transplant recipients. In regards to the detection of CMV DNA from blood, the specimens, WB or plasma, vary. Both specimens have been shown suitable for the monitoring of CMV infection in transplant recipients (4, 18, 19). A fully automated real time PCR assay, the IVD/CE-labeled Abbott RealTime CMV assay provides distinct procedures adapted to each of these specimens. Use of WB as a matrix, also convenient for other viral targets of interest in the monitoring of transplant
recipients (i.e. Epstein-Barr virus, Human herpes 6 virus), takes advantage for the laboratory workflow optimization by avoiding a centrifugation step required with plasma (20). In this study, we evaluated Abbott RealTime CMV assay for the quantification of CMV in WB.

CMV DNAemia dynamics should be taken into consideration for the initiation of preemptive therapy as underlined by recent studies (21, 22). Thus the reproducibility of the assay used for monitoring CMV infection is of great concern. In this regard the Abbott RealTime CMV assay was shown to achieve a high level of both intra and inter reproducibility with low coefficients of variation for high DNA levels and DNA levels close to the cut-offs usually proposed for therapy initiation. This low variability was further confirmed with the narrow range of Ct values of the IC supporting the effectiveness of the automated extraction procedure used in the assay. Importantly no PCR inhibitory effect was observed with the WB samples analyzed in the present study. Since the implementation of the assay in routine (from May 21st to September 30th 2012), IC cycle threshold was suggestive of PCR inhibitor or extraction deficiency in only 16 samples out of 4560 (0.35%) (i.e. cycle threshold value out of the range of validity as established automatically in the calibration run) (not shown).

The LLQ of the assay according to the manufacturer is 40 copies/ml of WB. This threshold was validated by testing QCMD panel samples and clinical samples diluted in WB to maintain extraction conditions, thus confirming the high sensitivity of this assays for CMV quantification in WB. This new commercial assay validated and released for WB samples reaches a higher sensitivity than the tests already commercially available which provide a higher LLQ (23, 24).

We compared Abbott RealTime CMV with Qiagen/Artus CMV assay on 302 clinical samples by using the same platform for both extraction and amplification, thus avoiding variability due to different instruments and extraction methods. The two assays were highly concordant. Similar kinetics of CMV replication from patients with successive samples confirmed this
correlation. Most of the discrepancies occurred for samples positive with the Abbott RealTime CMV assay and negative with the Qiagen/Artus CMV assay at low viral loads, which is in agreement with the lower limit of detection of the Abbott RealTime CMV assay validated with serial dilutions. Because the use of a third assay may have little chance to resolve the discrepancies, previous or subsequent samples collected within two weeks in the same patients were examined and all confirm that patients with discrepant results had a CMV active infection. The Abbott RealTime CMV assay showed a significantly higher sensitivity with 42.7% of samples positive compared to 35.4% with the Qiagen/Artus CMV assay. In addition, the Abbott RealTime CMV assay provided a 47.8% increase of quantified samples compared to the Qiagen/Artus CMV assay (32.8% versus 22.2%). This high sensitivity will allow to assess more precisely the kinetics of viral replication thus (i) to early detect individuals with short CMV doubling time and (ii) to better determine CMV half-time in patients receiving antiviral therapy. These measures could help to adjust the duration of treatment (3, 22, 25). Despite a good overall correlation among concordant positive results, differences above 0.5 log_{10} copies/ml were observed for 36.9% of these samples. Differences in DNA quantification techniques between laboratories have led to site-specific recommendations for both initiating and monitoring antiviral treatment. The international quantitative reference standard (WHO IS for CMV) currently available will likely allow direct comparisons between different quantification techniques after conversion of copies/ml into UI/ml. For the Abbott RealTime CMV assay, the manufacturer had determined the conversion factor in plasma and provides a unique conversion factor whatever the matrix used (WB or plasma). When using the WHO IS diluted in PBS or WB, the conversion factor experimentally determined in the present study was significantly different from the one proposed by the manufacturer. Its value was lower in PBS and higher in WB meaning that less CMV DNA was quantified from WB, likely reflecting to difference in DNA extraction.
efficiency. An even more divergent conversion factor for WB was found by Furione et al. using Abbott RealTime PCR assay and WHO IS diluted in WB (26). The reasons for this discrepancy were not clear. As the same automated extraction system was used in both studies, difference in extraction efficiency was very unlikely. The dilution factors of the reconstituted WHO IS in WB differed between the studies (1/16 in the study by Furione et al. versus 1/50 in the present study) (26). As indicated in the package insert, once reconstituted, the WHO IS should be diluted in the matrix appropriate to the material being calibrated. However, the dilution factor to apply is not indicated. Thus, in order to normalize the results obtained with different commercially available and in-house assays, dilution factors and the matrix used to dilute the WHO IS as well as the statistical analysis of the results should be standardized. Then, a detailed and consensual protocol should be defined to calculate the conversion factor according to assays and matrixes used.

In conjunction with current laboratory quality requirements as defined by the International Standardization Organization (ISO) 1589 norm for laboratory techniques, commercial assays fulfill several criteria that an individual hospital laboratory will achieve with difficulty. Considering the benefits of a fully automated assay in reducing hands on time and repetitive motion injuries and the traceability and security of blood sample management, and the precision and the reproducibility of the results observed in this study, Abbott RealTime CMV assay answers several quality requirements for the CMV quantitative assay implementation in a routine laboratory.

ACKNOWLEDGMENTS

We are grateful to Ghislaine Borit and Muriel Vitu for their technical assistance and Dr Nathalie Parquet for providing whole blood.
J. LeGoff, F. Simon and MC. Mazeron have consulted for Abbott Molecular. All other authors have declared that no competing interests exist. This does not alter the authors' adherence to all the Journal of Clinical Microbiology policies on sharing data and materials.
REFERENCES


15. Freyer JF, Heath AB, Anderson R, Minor PD, the Collaborative Study Group. 2010. Collaborative study to evaluate the proposed 1st WHO International Standard...
for human cytomegalovirus (HCMV) for nucleic acid amplification (NAT)-based assays. WHO ECBS Report 2010; WHO/BS/10.2138.


FIGURE LEGENDS

FIG 1 Bland Altman analysis between the CMV Qiagen/Artus and Abbott RealTime CMV assays for samples (n=65) with a CMV DNA load above the lower limit of quantification (LLQ) of each assay (2.30 log_{10} and 1.60 log_{10} copies/ml for the Abbott RealTime CMV and Qiagen/Artus CMV assays, respectively). CMV DNA loads are expressed in log_{10} copies/ml.

FIG 2 Kinetics of CMV replication in whole blood in seven patients, by using Qiagen/Artus CMV assay (open triangles) and CMV RealTime Abbott assay (closed circles). Patient 72 was an HSCT recipient and other patients were kidney transplant recipients. CMV DNA loads are expressed in log_{10} copies/ml.

FIG 3 Conversion factors (copies/ml to IU/ml) in PBS and whole blood. Eleven replicates of each dilution of the WHO IS in PBS or WB (at expected values of 1,000, 10,000 and 100,000 copies/ml) were tested in the same run. Boxplots of conversion factors estimated on each dilution were shown for PBS (A) and whole blood (B). Manufacturer’s values are given by the dotted horizontal line.
TABLES

TABLE 1 Lower limit of quantification (LLQ) of the Abbott RealTime CMV assay for whole blood.

<table>
<thead>
<tr>
<th>Expected values</th>
<th>No. of replicates</th>
<th>Mean values</th>
<th>Detection rate (%)</th>
</tr>
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<tbody>
<tr>
<td>QCMD&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
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<tr>
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<td>29</td>
<td>72</td>
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</table>

*Values are expressed in copies/ml.

<sup>b</sup> The LLQ was determined by using dilutions of QCMD CMV 10-08 sample in whole blood.

<sup>c</sup> The LLQ was determined by using dilutions of clinical samples in whole blood.

TABLE 2 Quantification of CMV DNA in QCMD 2010 CMV samples.

<table>
<thead>
<tr>
<th>QCMD sample</th>
<th>Expected values&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Abbott RealTime&lt;sup&gt;a&lt;/sup&gt;</th>
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</table>

<sup>a</sup> Values are expressed in log<sub>10</sub> copies/ml.

<sup>b</sup> nd: CMV DNA is not detected.
TABLE 1 Lower limit of quantification (LLQ) of the Abbott RealTime CMV assay for whole blood.

<table>
<thead>
<tr>
<th>Expected values</th>
<th>No. of replicates</th>
<th>Mean values</th>
<th>Detection rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>QCMD&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>30</td>
<td>91</td>
<td>100.0</td>
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<tr>
<td>150</td>
<td>30</td>
<td>67</td>
<td>100.0</td>
</tr>
<tr>
<td>100</td>
<td>28</td>
<td>39</td>
<td>89.3</td>
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<tr>
<td>50</td>
<td>30</td>
<td>20</td>
<td>70.0</td>
</tr>
<tr>
<td>25</td>
<td>30</td>
<td>22</td>
<td>20.0</td>
</tr>
<tr>
<td>Clinical samples&lt;sup&gt;c&lt;/sup&gt;</td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>30</td>
<td>147</td>
<td>100.0</td>
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<tr>
<td>100</td>
<td>30</td>
<td>142</td>
<td>100.0</td>
</tr>
<tr>
<td>50</td>
<td>29</td>
<td>72</td>
<td>96.7</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values are expressed in copies/ml.

<sup>b</sup>The LLQ was determined by using dilutions of QCMD CMV 10-08 sample in whole blood.

<sup>c</sup>The LLQ was determined by using dilutions of clinical samples in whole blood.
TABLE 2 Quantification of CMV DNA in QCMD 2010 CMV samples.

<table>
<thead>
<tr>
<th>QCMD sample</th>
<th>Expected values a</th>
<th>Abbott RealTime CMV a</th>
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<tbody>
<tr>
<td>10-01</td>
<td>3.27</td>
<td>2.96</td>
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<td>10-02</td>
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<td>10-03</td>
<td>5.44</td>
<td>5.23</td>
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<td>10-04</td>
<td>3.74</td>
<td>3.45</td>
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<tr>
<td>10-05</td>
<td>nd\textsuperscript{b}</td>
<td>nd\textsuperscript{b}</td>
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<tr>
<td>10-06</td>
<td>6.41</td>
<td>6.15</td>
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<tr>
<td>10-07</td>
<td>3.26</td>
<td>3.03</td>
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<td>10-08</td>
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<td>10-09</td>
<td>2.84</td>
<td>2.59</td>
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<tr>
<td>10-10</td>
<td>4.23</td>
<td>4.07</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Values are expressed in log\textsubscript{10} copies/ml.

\textsuperscript{b} nd: CMV DNA is not detected.