VALIDATION OF A COMMERCIAL REAL-TIME QUANTITATIVE PCR ASSAY FOR DETECTION OF CMV VIRAL LOAD IN PLASMA OF TRANSPLANTATION RECIPIENTS AND ASSESSMENT OF WHO CMV INTERNATIONAL PANEL FOR IN-HOUSE CMV ASSAY CALIBRATION

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STUDY BACKGROUND AND AIM
Monitoring cytomegalovirus (CMV) viral load in the plasma provides essential information for the management of CMV disease in post-transplant patients. An in house real time PCR assay (LOD-QPCR) has been implemented at ProvLab for detection of CMV viral load in plasma (reporting in copies/ml) since 2003. Several commercial assays are now available for measuring CMV viral load with improved QC. The variability between the molecular methods employed, and the lack of traceability to a reference system, make it difficult to compare assay performance and to develop uniform treatment strategies. The first World Health Organization (WHO) International Standard for human cytomegalovirus (HCMV), NIBSC code 09/162, is now available to be used in the standardization of nucleic acid amplification technique (NAT)-based assays for HCMV. The purposes of this study were:

1. To determine the linearity, a series of 10-fold dilutions of CMV Merlin strain (Sample 1), AD169 strain (Sample 2) and a clinical PL specimen of high VL, and AD169 strain from cell culture.
2. The specificity of each assay was confirmed with Epstein-Barr virus (EBV), varicella-zoster virus (VZV), herpes simplex virus (HSV), BK virus, and adenovirus. The DNA of each virus was run in duplicate. Both assays were specific and no cross-reaction was observed between CMV and the other viruses (Data not shown).
3. To investigate assay to assay correlation and to look at inter-lab and inter-instrument variation, 50 DNA PL specimens were run using both assays at both ProvLab sites.
4. Precision determination was performed using 3 DNA samples run in triplicate over 5 days using the same instruments.
5. To determine the accuracy of the quantitation, 100 DNA PL specimens were run using both assays at both molecular diagnostic labs in ProvLab and run on both assays.

RESULTS AND DISCUSSION

OBJECTIVE 1: RealStar™ Validation against LOD-QPCR

1. Comparative limit and linearity of CMV detection was observed between LOD-QPCR and RealStar™ QPCR. The variation was greater for both assays when the VL was low. The VL for the LOD-QPCR was greater in absolute number than the RealStar™ QPCR. However, the RealStar™ QPCR assay was more sensitive and detected approximately 1.0 log more than the LOD-QPCR.

2. Inter-laboratories (A) and -instruments (B) variations were evaluated using 50 DNA calibrators used in each assay. With the new WHO CMV International Standard, this could introduce error will have a significant effect. A WHO international reference standard for CMV-DNA050 and extracted, the calibration of the LOD-QPCR/RealStar™ by a factor of about 2-fold. Note that the LOD-QPCR/RealStar™ conversion factor of LOD-QPCR to RealStar™ assay was approximately 2-fold.

3. The precision was evaluated using three DNA samples tested in triplicate over five different days using the same instrument to minimize inter-instrument variation. A precision of CV = 1.44 ± 0.35 % and a narrow variation of inter-laboratories and instruments were observed in RealStar™ QPCR.

OBJECTIVE 2: Calibration of LOD-QPCR with the WHO CMV International Standard

Using WHO HCMV (IU/ml) that was diluted in BaseMatrix™ and extracted, the calibration of the LOD-QPCR assay was determined as 5.65 copies/ml to 1 IU/ml.

A direct comparison of the two assays using the WHO HCMV reconstituted as per the product insert and extracted, AD169 DNA, and extracted clinical PL samples confirmed an overall conversion of LOD-QPCR/RealStar™ by a factor of about 2-fold. Note that the LOD-QPCR/RealStar™ using three DNA samples assigned as 0 in BM IU/ml, and extracted, the calibration of the LOD-QPCR/RealStar™ conversion factor of LOD-QPCR to RealStar™ assay was approximately 2-fold.

CONCLUSIONS

The precision, sensitivity, linearity and the use of an internal control make the RealStar™ QPCR a very good alternative assay for monitoring CMV VL in post-transplant patients. However, even with a commercial kit, quality control and monitoring are still essential as any introduced error will have a significant effect. A WHO international reference standard for CMV VL assay calibration is an important step in quality improvement and inter-lab communication. In our lab, the LOD-QPCR calibration is 5.65 copies/ml to 1 IU/ml.

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