Results of the first French National Quality control for CMV viral load in whole blood

Sophie Alain [1,2]
1 : French National reference center for Cytomegaloviruses, CHU Dupuytren, Limoges France
2 : Bacteriology –Virology-Hygiene department, CHU Dupuytren, Limoges, France
3 : Argene Corporation, Varhiles, France
sophie.alain@unilim.

Introduction

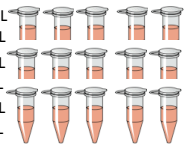
The large use of Cytomegalovirus (CMV) viral load quantification to follow immunosuppressed patients need standardized assays. Whole blood allows follow-up for several viruses and simplifies pretreatment and storage of samples. However, sensitivity and range of quantification may vary with the extraction method or amplificatio assay. The French national reference center for Cytomegaloviruses first organized an investigation of practice in 37 teacher hospital virology laboratories to assess the situation in France in 2010. 36/37 laboratories filled the questionnary. Among these, 67% used the quantitative PCR in routine, 11% antigenemia and 22% antigenemia or quantitative PCR; 87% of the laboratories use whole blood for quantitative PCR, whereas 10% and 3% use plasma and leukocytes respectively. Among the laboratories using DNAemia, 100% used real-time PCR assays, 91% use an automated extraction and 9% a manual extraction. Thus in France, measurement of DNAemia by real-time PCR is a tool which gradually replaces the antigenemia for the monitoring of cytomegalovirus infection among immunocompromised patients. The very great diversity of the methods used justifies the installation of a national quality control on total blood, matrix used by 87% of the laboratories.

Materials and Methods

Pending an international standard, our reference laboratory performed a national quality control (QC) in **24 laboratories** using a high titered saliva sample (8logcopies/mL) diluted in whole blood.
The panel contained 7 blood samples (N° 1 to 7) : one negative and 3 CMV-positive with two aliquotes per point. They were tested blindly bty the laboratories. The volume was sufficent for testing each point of

=

10^E3 copies/mL
3log copies/mL
10^E4 copies/mL
4 logcopies/mL
10^E6 copies/mL
6log copies/mL



Stored at -80°C
Tested in 4 PCR assays : in-house PCR within *UL83* and *UL123* genes Artus® CMV LC PCR Kit (QIAGEN) and R-gene™(ARGENE)

Validation of the three points:
54 tests for each point (*UL83*: 14, *UL123*: 6, Rgene™: 19, artus® CMV: 15)

CMV-positive panel target values :
QCLow=QCL (N°s 5 et 6) : 1,97 log copies/mL (ET 0.78, median 2,10)
QC Medium=QCM (N°s 3 et 4) : 3,15 log copies/mL (ET 0.80, median 3.33)
Point théorique 10^E6 QC High=QCH (N°s 1 et 2) : 5,17 log copies/mL (ET 0.90 median 5.52)

These target value are under the theorical values, because they represent the mean of 4 different PCR assays

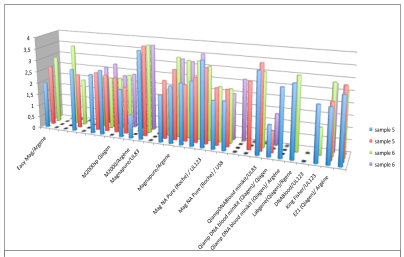
Results

Specificity : 100% The negative sample was negative in all laboratories

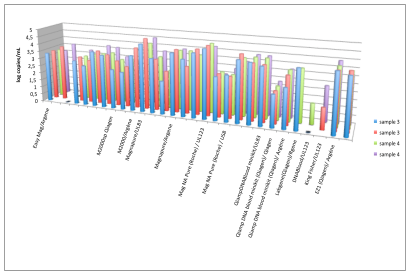
Sensitivity and viral load quantification : The QC low (1,97 log copies/mL) is not detected by all methods, The other points are all detected , in duplicate, though viral load may vary greatly between methods. In house assays proved less sensitive..

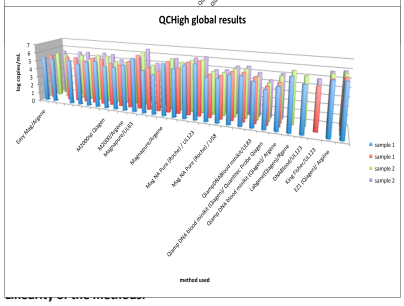
Details of results : Each row represent one method. For methods used by several laboratories the method name is in front of the first laboratory. For Easy Mag (BioMérieux) /CMVRgene(Argene) and for Magnapure (Roche)/US8 (In House) the two laboratories tested the panel in duplicate.
Values: QCL(only detected by 19 laboratories) mean 2,10 SD 0,78 **QCM:** mean 3,33 SD 0,80 **QCH:** mean 5,52 SD 0,90. CMV Artus with manual extraction and US8 PCRs underscored the QCH and QCM.

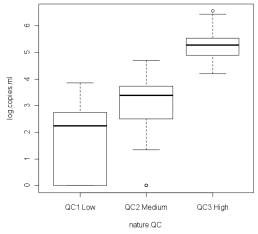
QC Low :



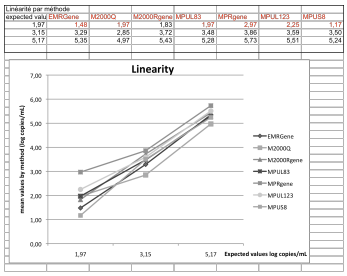
QC Medium :

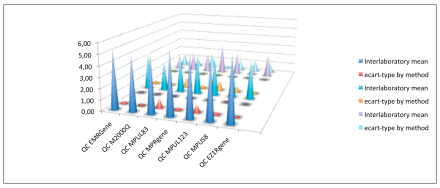






. Interlaboratory variability was wide for QCL and QCM (CV 13 to 125%), low for QCH (CV 1 to 16%), major for in house assays whatever the extraction





. **Intralaboratory variability** was under 0.3logs and 0.4logs for QCH and QCM except for one laboratory and from 0.3 to 1.6logs for QCL.

Conclusion

This quality control shows the great specificity of CMV PCR quantification assays, whatever the method, without any contamination, and the variability of methods linked both to the PCR assay (with under quantification by some assays) and to the extraction method. Though CE marked assays show better reproducibility than in house assays, extraction may influence the results, particularly on the whole blood matrix. This demonstrate the usefulness of an international standard and the need to follow patients with the same assay.