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Comparison between the LightCycler® CMV Quant Kit (Roche Diagnostics) with a standardized in house Taqman assay for cytomegalovirus blood viral load quantification

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Introduction

The interest in monitoring of CMV viral load in immunocompromised patients justifies the development of real-time PCR assays. However the choice of the matrix and the method will influence the quantification results. In-house methods are numerous and several commercial kits are available for routine monitoring of transplant recipients. The use of whole blood, which simplifies the pre-analytic step and allows combined research for many viruses of importance in transplantation (EBV, HHV6, BKV) associated with a standardized kit could be an ideal choice, which requires validation of the kit with whole blood.

The LightCycler ® CMV Quant kit has been previously approved in France for CMV quantification from plasma. We therefore aimed to evaluate the performance of the kit LightCycler® CMV Quant kit (Roche Diagnostics) on whole blood, when used on the Light Cycler 2.0 apparatus, compared to an in-house Taqman assay amplifying a fragment of *UL83* validated and used in routine laboratory of the National Reference Center of CMV, known as LC1 UL83PCR (Mengelle et al., 2003).

Materials

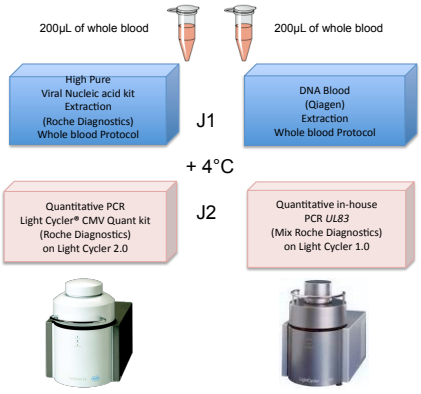
Plasmatic Quality Control
- pure or diluted in whole blood before extraction
- panel used for the European Quality Control QCMD, distributed by Argène Biosoft, France

Range of CMV culture supernatants
AD169 strain diluted in CMV seronegative whole blood

47 samples of whole blood
from transplant recipient analyzed by both PCR assays

All controls or samples were simultaneously analyzed by both assays

Methods



Light Cycler® CMV Quant kit:
✓Scorpion method with UDG
✓Internal control added during extraction
✓Used on Light Cycler® 2.0
✓Manual extraction with High Pure microcolumns
✓CE label for CMV quantification in plasma

In-house assay:
✓Taqman assay with UDG (Mengelle et al., J. Med Virol, 2003)
✓Used on Light Cycler® 1.0
✓No internal control: external control with albumin PCR (Wagner et al., J. Virol. Methods, 2007)
✓Manual extraction with Qiagen microcolumns
✓Technically and in clinical practice validated
✓Used in many laboratories

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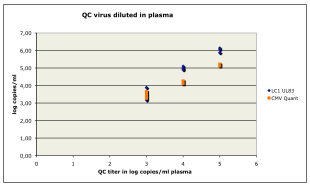
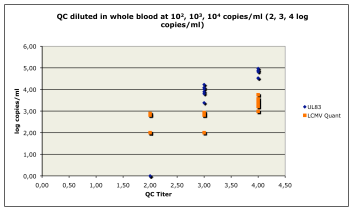
Results of quality control

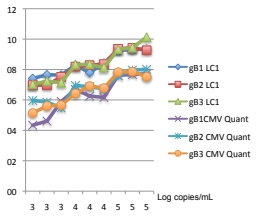
- **Plasmatic quality control:**
  - Virus diluted in plasma: 10^3, 10^4 and 10^5 copies/mL ⇔ 3, 4, 5 log copies/mL, 6 points per titer
  - R^2 = 0,88
  - Mean difference between methods : 0,55 log, SD : 0,42



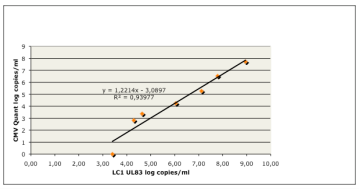
- **Whole blood quality control:**
  - LC1 UL83 : 6 points, CMV Quant : 12 points
  - Extraction reproducibility checked with albumin PCR
  - Mean difference: 0,05 log, SD 1,94 (mean :1,35 log from 3 logs)



- **No influence of genotype on quantification :**
With suspensions of cells infected by genotype gB 1,gB2 or gB3 isolates titered at 10^3, 10^4 and 10^5 UFCI in culture

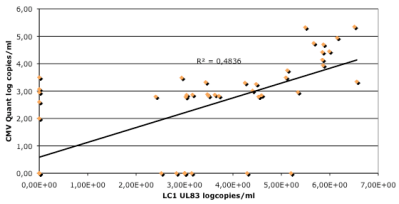


- **Quality control with strain AD169 diluted in whole blood for values above 3log copies/mL:**

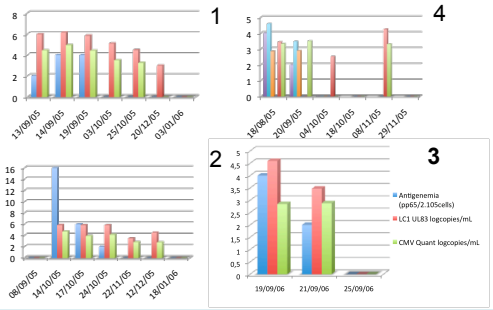


Results of clinical samples

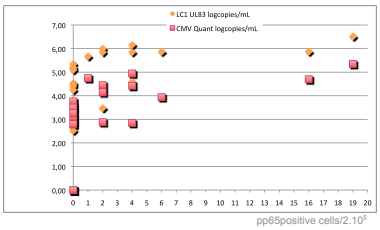
- **47 samples from transplant recipients**
  - Mean difference between both methods : 0,76 log, SD : 1,69
  - Correlation and trend curve :



- **Correlation with pp65 antigenemia in virological follow-up of 4 patients**



- **Correlation with weak values of pp65 antigenemia**



Conclusion

Quality Controls: The results of both methods are well correlated with the values expected for the plasmatic quality control, with a coefficient R^2 of 0.88. On whole blood, the overall correlation is poor (R^2 0.45): there is a sub-quantification below 3 logs and an over-quantification in high-range values with LC1 UL83 PCR. Above 3 logs correlation is excellent R^2 0.93, with a 1.2 log gap of over-quantification with LC1 PCR. The results of CMV Quant are close to expected values for quality control.

Clinical samples: A 0.76 log copies / mL mean difference of quantification is observed in these samples. The results are comparable to those of quality control. gB genotype, as expected, does not alter the results.

Correlation with pp65 antigenemia: Tested only on low values, the correlation is poor, with major discrepancies for some patients (4).

In summary:
CMV Quant assay is reproducible and shows a wide range of linear quantification on whole blood. It confirms on whole blood its performance in plasma and can therefore be recommended for this indication. The results of quality controls on whole blood underline the need for a standard range for this type of matrix. Differences between both methods justify to follow a patient with the same technique throughout his clinical history.