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Evaluation of CMV R-gene PCR (Argene) coupled with EasyMag Biomérieux extraction for CMV viral load quantification in amniotic fluid

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Introduction

Diagnosis of Cytomegalovirus (CMV) congenital infection *in utero* relies on viral DNA detection in amniotic fluid. Though many PCR methods have been applied to CMV viral load measurement, only few of them are currently validated for antenatal diagnosis. In particular, extraction methods, which can influence both reproducibility and sensitivity of PCR in such cellular-rich fluids, have to be cautiously analysed. We herein aimed to study the performances of the CMV R-gene PCR kit (Argene, France), CE marked for AF with Qiagen DNA blood and M2000 Abbott extraction methods, and used here with the automated EasyMag extraction (BioMérieux, France) method.

Materials

• Positive samples for comparison of methods and reproducibility after conservation

- 2 CMV-negative amniotic fluids spiked with high titer saliva sample (10^e8 copies/ml) N° 506 and N° 322
- 2 CMV-positive amniotic fluids diluted in negative amniotic fluid N° DEM and N° K.

• Positive control: International Standard diluted in negative amniotic fluid from 6,7 to 0,7 log copies/mL.

• CMV-negative samples for specificity (not diluted)

- 6 negative amniotic fluids.
- 5 positive amniotic fluids (2 for Parvovirus B19, 1 for HSV1, 1 for Enterovirus, 1 for HHV6).

All these samples were kept frozen at -80°C before extraction.

Methods

All samples and dilution were extracted and tested in parallel with both PCR combined with each extraction method.

EasyMag:
Sample: 200µl
Pre-lysis with PK at 56°C
Protocol B
Elution 100uL

M2000sp:
Sample: 200µl
Elution: 100µL

DNA blood Qiagen:
Sample: 200µl
Manual minicolumn extraction
Elution 100µL

10µL

Quantitative real-time PCR assay

In-house UL83 assay:

✓Taqman assay with UDG (Mengelle et al., J. Med Virol, 2003)
✓Light Cycler® 1.0 (Roche)
✓external control with albumin PCR (Wagner et al., J. Virol. Methods, 2007)
✓Technically and in clinical practice validated
✓Used in many laboratories until 2010

CMV Rgene assay:

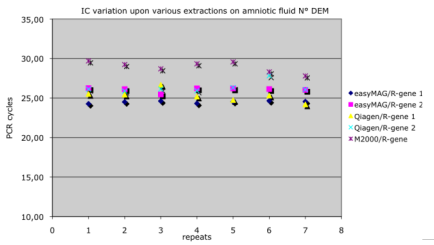
✓Taqman assay
✓Rotor gene apparatus
✓Internal control in multiplex
✓CE marked for amniotic fluid with Qiagen DNA blood and M2000 Abbott extraction methods

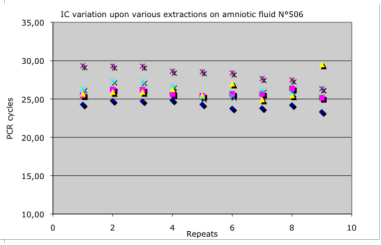
Results

Specificity: 100% all the samples were negative for CMV with both assays.

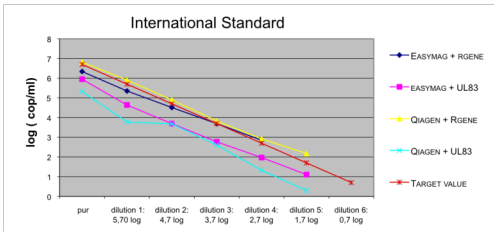
Reproducibility was tested on the internal control: Internal control values were highly reproducible with the R-gene assay

CMV-negative samples: mean value: 27.05 +/-0.46 cycles
Positive control: 24.06 +/-0.18 (EasyMAG bioMérieux) versus 27.54 +/-2.27 cycles (DNA blood Qiagen)
Positive samples: 28.54 +/-0.82 (Abbott M2000sp), 24.48 +/-0.41 ; 25.95 +/-0.38 (EasyMAG bioMérieux), 26.3 +/-1,.; 26.50 +/-0.68; (DNA blood Qiagen).





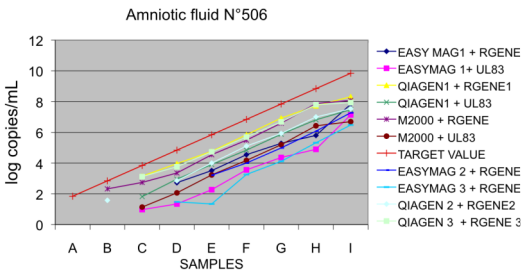
Impact of extraction method on CMV quantification

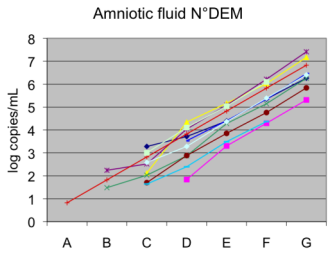


• Viral load quantification was lower with the UL83 than with R-gene assay whatever the extraction method and the positive sample or standard.

• From the International standard we observe an excellent correlation between EasyMAG extraction and manual DNA blood extraction with both methods (R^2= 0.99 for Rgene and 0.94 for UL83). EasyMAG values were slightly higher than Qiagen values for both methods (mean 0.32+/-0.1 for R-gene).

• The lower viral load samples were consistently detected at 3 log copies/mL in samples and in the standard but not at lower values





Conclusion

CMV R-gene internal controls were highly reproducible with Easy Mag showing the good performance of the extraction method. On diluted samples and on the standard the four combinations show good correlation and a high linearity from 6 to 2 log without any saturation effect for highest viral loads.
Quantification results between easyMAG extraction and manual DNA blood and M2000sp are reliable. Quantification of CMV in amniotic fluid with CMV R-gene™ after easyMAG extraction can be performed.
The three combinations of CMV R gene assay with either manual DNA blood, EasyMAG or M2000sp are reliable for CMV load measurement in amniotic fluid, though these results have to be confirmed on a panel of CMV-positive undiluted AF.

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