Comparison of QIASymphony® (QIAGEN) and Easy Mag® (BioMérieux) for human cytomegalovirus DNA extraction from total blood.

P 157630

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Introduction

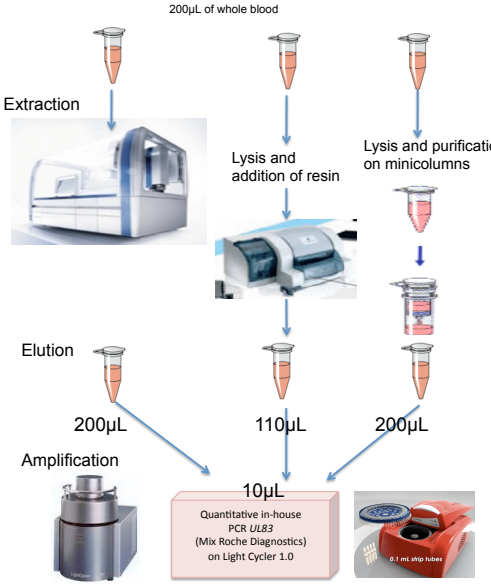
Cytomegalovirus viral load quantification with real-time PCR is widely used in immunocompromised patients. Total blood is largely used in France for follow-up of multiple viruses involved in post–transplant diseases. Preanalytic phase is simplified and several PCRs can be performed from a unique extract. Though, extraction step needs to be standardized, which is proposed by several manufacturers through automated extraction.
We evaluated two automated extractions on magnetic silica particles with a panel obtained from dilutions of a highly titer saliva sample in whole blood (10^e8 copies/ml) and clinical whole blood samples of low viral load. QIAsymphony (QIAGEN) is completely automated, from primary tube to eluted DNA. Easy Mag (BioMérieux) is automated from lysis to elution. Manual extraction with QIAamp DNA blood (QIAGEN) with same sampling volume (200µL) was used as a reference.

Materials

- **Quality Control**
-High titer saliva sample (10^e8 copies/ml) diluted in whole blood from 10E6 to 10 copies/mL before extraction
- **14 routine samples of whole blood of low viral load**
- **All controls or samples were simultaneously analyzed by both assays**

•To see if the use of saliva as a quality control can impact the resuts we also tested 17 saliva samples obtained from children in a storage medium with the Oragene® sampling kit (DNA Genotek, Canada) and previously titered after manual extraction with DNA blood.

Methods



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

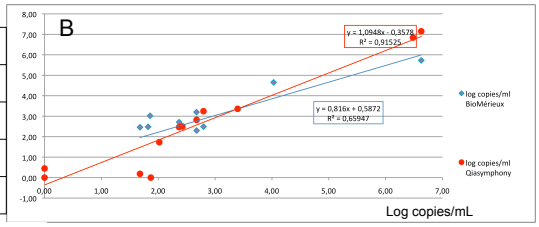
Results on the whole blood quality control and on saliva

Saliva :
A : Saliva is stable after dilution and conservation and can be used to produce quality controls
B : Extraction does not modify the results on saliva

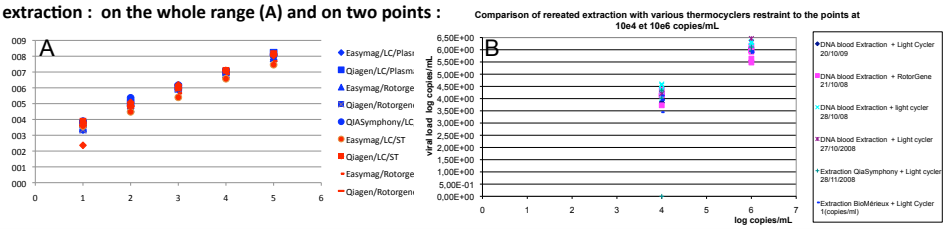
A

Assays on dilutions	DNA blood extraction + LightCycler 1 (copies/ml)	Mean
10e4 +4°C n°1	9,723E+03	
10e4 +4°C n°2	6,684E+03	1,101E+04
10e4 +4°C n°3	1,662E+04	
10e4 -20°C n°1	8,600E+03	
10e4 -20°C n°2	1,413E+04	8,380E+03
10e4 -20°C n°3	2,410E+03	
10e6 +4°C n°1	5,421E+05	
10e6 +4°C n°2	9,922E+05	8,099E+05
10e6 +4°C n°3	8,953E+05	
10e6 -20°C n°1	7,303E+05	
10e6 -20°C n°2	7,124E+05	6,933E+05
10e6 -20°C n°3	6,371E+05	

B



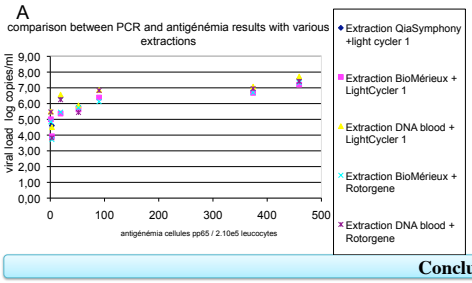
Whole blood
extraction : on the whole range (A) and on two points :



Results of clinical samples

Routine samples from transplant recipients :
A : 7Samples of known viral load measure by antigenaemia were simultaneously tested with the three extraction methods and Tested with either a Light Cycler 1 or a Rotor Gene thermocycler and **B** : 11 samples of low viral load

A



B

	DNA bloodExtraction + LightCycler 1 (copies/ml)	Extraction Biomérieux + LightCycler 1 (copies/ml)	Extraction Biomérieux + LightCycler 1 (copies/ml)	Extraction Biomérieux + Rotorgene (copies/ml)	Extraction Biomérieux + Rotorgene (copies/ml)
080015645	0	1730	1421	63	1207
080022819	625	502	0	115	0
080018059	835	0	0	0	220
080025272	1472	296	0	815	0
080025486	2597	1260	1290	4572	4539
080023025	1,832E+06	1,755E+06	1,228E+06	7,717E+05	3,221E+05
080025308	0	0	0	0	0
080025344	0	0	0	0	0

Conclusion2

Quantification after extraction of the panel (10^e6 à 10 copies/mL) with the three methods never differ from more than 0,5log. Though points 10 et 10^e2 were not detected. Reproducibility (tested on points 10^e4 et 10^e6) consistenly shows differencies less than 0,5log. Tested on 11 samples of low viral load (from 10^e2 to 10^e4): 6/6 were concordant with QiaSymphony, and 3/5 with Easy Mag.

In Conclusion : These results show 1) the feasability of a quality control from saliva diluted in whole blood and
2) the good performances of both automated extractions on CMV DNA from whole blood, whatever the viral load, particularly at the therapeutic threshold of 10^e4copies/mL.

Acknowledgements: Qiagen and Bio Mérieux (France) for providing the extractors and the kits