Comparative evaluation of the NucliSENS® easyMAG™ automated system for the extraction of viral DNA from whole blood samples: application to the monitoring of cytomegalovirus (CMV) and Epstein-Barr virus (EBV) loads

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Background

Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) infections represent significant clinical problems for immunocompromised patients: primary infections as well as reactivations cause morbidity, prolong hospitalisation of the patients and increase the cost of health care. CMV infection is also a negative prognostic factor for renal graft.

Frequent measuring of CMV and EBV viral loads permits the installation of pre-emptive treatment and the monitoring of treatment efficiency. Whole blood is now recognised as the best sample for CMV and EBV viral loads quantification in immunocompromised patients [1, 4, 6].

Due to the high amount of genetic material in whole blood samples, new extraction methods must be carefully evaluated by comparison to the reference techniques such as those using columns to recover correctly the nucleic acids used in amplification techniques.

Objective

To evaluate the ability of the NucliSENS® easyMAG™ automated system (bioMérieux) using magnetic silica to extract viral nucleic acids from human whole blood for the determination of the viral load in patients suspected of CMV or EBV infection.

Materials & Methods

• **Whole blood samples collected in patients with suspected CMV infection:**
  - Selection of positive and negative CMV samples using an in-house real time PCR [5]
  - Selection of positive and negative EBV samples using a commercial technique (LightCycler EBV Quantification kit, Roche Diagnostics)
  - For CMV analysis: 96 samples including 75 found initially positive
  - For EBV analysis: 80 samples including 59 found initially positive
• **Comparison of 2 methods of DNA extraction:**
  - Reference manual method: columns of QIAamp® DNA blood extraction kit (Qiagen)
 - New automated method: Specific B protocol on NucliSENS easyMAG instrument (bioMérieux)
• **CMV and EBV quantification:**
  - Using respective R-gene® amplification kits (Argene Biosoft) and ABI 7500 instrument (Applied Biosystems). Both kits have been previously validated for quantification of CMV and EBV loads in whole blood [2, 3]
  - Results validated via inhibition controls and expressed in viral copies/ml of whole blood
• **Statistical analysis:** comparison of qualitative results obtained with both methods, expressed as percent agreement, and comparison of quantitative results for positive samples (Bland-Altman graphs)
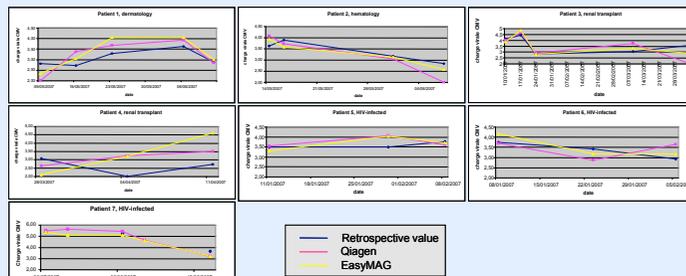
Specific B protocol
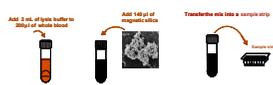
Results

		Qiagen		
		+	-	total
NUCLISENS easyMAG	+	61	8	69
	-	4	22	26
	total	65	30	95

		Qiagen		
		+	-	total
NUCLISENS easyMAG	+	59	8	67
	-	3	5	8
	total	62	13	75

Figure 1: Qualitative analysis of CMV detection after extraction using columns or automated method.
A- Analysis of the 95 specimens (one sample found inhibited after easyMAG extraction was excluded from the analysis). The overall agreement is 87.4% [79.0 - 93.3%].
B- Analysis of the 75 specimens found initially positive. The percent positive agreement is 89.3% [80.1– 95.3 %] for NUCLISENS easyMAG extraction and 82.7% [72.2 - 90.4%] for extraction with Qiagen columns.
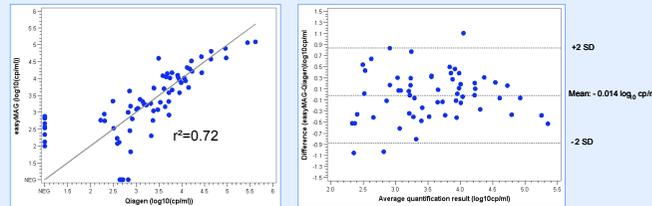
Figure 2: Quantitative analysis of CMV detection after extraction using columns or automated method.
A- Comparison of the 60 specimens found positive after extraction by both methods. The gray line shows the line of identity (X=Y).
B- Bland-Altman plot. Each plot represent the difference observed between the results of the 2 methods against their mean. The mean of difference is -0.014 [-0.125; +0.096]. The standard deviation (SD) is 0.427.


Figure 3: Follow-up of viral load in 7 CMV infected patients.

		Qiagen		
		+	-	total
NUCLISENS easyMAG	+	52	15	67
	-	0	13	13
	total	52	28	80

		Qiagen		
		+	-	total
NUCLISENS easyMAG	+	49	10	59
	-	0	0	0
	total	49	10	59

Figure 4: Qualitative analysis of EBV detection after extraction using columns or automated method.
A- Analysis of the 80 specimens. The overall agreement is 81.3% [71.0 - 89.1%].
B- Analysis of the 59 specimens found initially positive. The percent positive agreement is 100% [93.9 – 100%] for NUCLISENS easyMAG extraction and 83.1% [71.0 - 91.6%] for extraction with Qiagen columns.
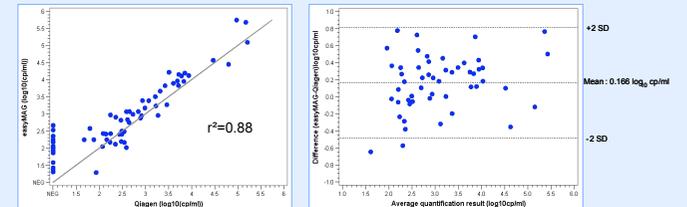
Figure 5: Quantitative analysis of EBV detection after extraction using columns or automated method.
A- Comparison of the 52 specimens found positive after extraction by both methods. The gray line shows the line of identity (X=Y).
B- Bland-Altman plot. Each plot represent the difference observed between the results of the 2 methods against their mean. The mean of difference is 0.166 [+0.076; +0.256]. The standard deviation (SD) is 0.324.

The total percent agreement between the two extraction techniques was of 87.4% for CMV and 81.3% for EBV. The percent positive agreement of the NucliSENS easyMAG extraction method compared to routine status was 89.3% and 100% for CMV and EBV, respectively; by comparison, the Qiagen extraction method exhibited a percent positive agreement of 82.7% and 83.1% for CMV and EBV, respectively.
 For samples found positive after extraction by both methods, the correlation coefficient (r test) between the viral loads was $r^2=0.72$ for CMV and $r^2=0.88$ for EBV; the mean difference in viral loads was -0.014 log copies/mL for CMV (not statistically significant) and 0.166 log copies/mL for EBV to the benefit of the automated extraction method (p<0.001).

Discussion

The excellent concordance between the results obtained after using both extraction methods validates the capacity of the "Specific B" protocol to extract viral DNA from whole blood with the NucliSENS easyMAG system in two models measuring viral load. A trend was noted for a better sensitivity of NucliSENS easyMAG regarding the low values. The additional advantages of this automated extraction technique include:
  - reduction of technical time (2/3)
  - reduction of cross-contamination avoiding centrifugation,
  - improvement of standardisation,
  - traceability
  - quality control assessment.

References

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Acknowledgments