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, prolonging hospitalisation of the patients and increasing the cost of health care. CMV infection is also a negative prognostic factor for renal graft.

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.

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 (LHHC)by 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 method: column of Qiagen® 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 Biocine) 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)

Results

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 to the NucliSENS easyMAG 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) between the viral loads was r=0.72 for CMV and r=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 (23%)
- Reduction of cross-contamination avoiding centrifugation
- Improvement of standardisation
- Traceability
- Quality control assessment

Conclusion
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:

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References
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