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A new highly automated extraction system for quantitative real-time PCRs from whole blood samples: Routine monitoring of opportunistic infections in immunosuppressed patients

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ABSTRACT

Background: Rapid, high throughput extraction systems are needed to monitor viral infections in immunosuppressed patients.
Objectives: Evaluate the performance of the MagNA Pure 96™ extraction system, and compare it to the COBAS Ampliprep™ for quantitative real-time PCR from whole blood samples.
Study design: Compare the MagNA Pure LC™, COBAS Ampliprep™ and MagNA Pure 96™ using ten-fold dilutions of blood samples containing cytomegalovirus. Evaluate analytical performances of the MagNA Pure 96™ from test samples containing cytomegalovirus. Evaluate clinical performances from 209 blood samples collected prospectively, extracted with the COBAS Ampliprep™ and the MagNA Pure 96™ systems and tested for cytomegalovirus, Epstein–Barr, BK and JC viruses.
Results: All three extraction systems gave similar results with dilutions of a cytomegalovirus-positive sample. Analytical tests showed that the limit of detection was 500 copies/ml, specificity was 100%, with no cross-contamination. Quantification was linear from 3.0 to 6.0 $\log_{10}$ copies/ml. Intra-assay variation was 8.3–0.9% and inter-assay variation 8.8–5.2%. Clinical specimens extracted with the MagNA Pure 96™ and COBAS Ampliprep™ instruments agreed well for cytomegalovirus ($r = 0.54$; $p = 0.07$), Epstein–Barr virus (0.69; $p = 0.0005$) and BK virus (0.85; $p = 0.01$). All 55 samples were negative for JC virus. Mean loads were similar for cytomegalovirus (0.17 $\log_{10}$ copies/ml) and BK virus (−0.24 $\log_{10}$ copies/ml) while that of Epstein–Barr virus was slightly lower (1.02 $\log_{10}$ copies/ml).
Conclusions: The MagNA Pure 96™ instrument is an easy-to-use, reliable high throughput platform for extracting nucleic acid from clinical whole blood specimens.

1. Background

Opportunistic infections among transplant patients due to viruses such as those of the herpesviridae and polyomaviridae families can be surveyed and controlled by iterative blood sample collection.[1,2] But this, in turn, has dramatically increased the number of routine tests performed by virology departments signalling the need for fully automated extraction instruments.

Currently plasma samples are used routinely for quantifying HIV,[3,4] HCV,[5] and HBV virus nucleic acids with high throughput automated platforms.

The samples used for molecular assays of other viruses such as cytomegalovirus (CMV), Epstein–Barr virus (EBV), and BK virus may vary from serum and plasma to whole blood, depending on local preference. Whole blood samples are the most popular in France, but their extraction has yet to be completely automated. In this context some high throughput automated platforms have been tested: the NucliSENS easyMAG system (bioMérieux, The Netherlands) was compared to the column-based Qiagen method for extracting CMV-DNA.[6] More recently, the extraction of CMV and EBV DNA by the *m*1000 system (Abbott Laboratories, Illinois) was compared to the QIAamp UltraSens virus kit (QIAGEN, Hilden, Germany)[7] systems.

While all three of these platforms are convenient for extracting high quality virus nucleic acids, they remain time-consuming and require two to four hours to extract 96 samples.

Abbreviations: IQC, intra-laboratory quality control; QCMD, Quality Control for Molecular Diagnosis; CMV, cytomegalovirus; EBV, Epstein–Barr virus; HIV, human immunodeficiency virus; HCV, Hepatitis C Virus; HBV, Hepatitis B Virus; HSV, herpes simplex virus; SD, standard deviations; CV, coefficient of variation.

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2. Objectives

The MagNA Pure 96™ (Roche Diagnostics, Meylan, France) became available recently. It appears to be very rapid, with 96 samples extracted in less than 1 h. We therefore analysed its performance and its convenience in routine use for extracting virus nucleic acids from whole blood specimens from immunosuppressed patients collected in the course of a single week. Its performance was compared to that of the COBAS Ampliprep™ instrument (Roche Diagnostics, Meylan, France).

3. Study design

3.1. Materials

3.1.1. Material used to compare the MagNA Pure LC™, COBAS Ampliprep™ and MagNA Pure 96™

An intra-laboratory quality control (IQC) was prepared by pooling known cytomegalovirus (CMV) positive whole blood clinical samples and was diluted ten-fold with negative whole blood. Each dilution was extracted twice with each of the three extraction systems.

3.1.2. Material used for the MagNA Pure 96™ study

3.1.2.1. Sensitivity. A commercial human cytomegalovirus sample from the Quality Control for Molecular Diagnosis (QCMD) (Glasgow, Scotland) was tested five times in the same run.

3.1.2.2. Specificity and cross-contaminatons. 20 CMV-negative samples were tested. They included 3 samples containing herpes simplex virus (HSV) and 3 samples containing Epstein–Barr virus.

Lack of cross-contaminations was tested by extracting alternating high positive ($n = 3$) and negative ($n = 3$) samples.

3.1.2.3. Linearity. Serial ten-fold dilutions (in CMV-negative whole blood) of a clinical sample with a high CMV load were tested.

3.1.2.4. Intra-assay and inter-assay reproducibility. Intra-assay variation was assessed on seven QCMD samples positive for CMV (range 3–7 $\log_{10}$ copies/ml). Each sample was tested three times in the same run.

Inter-assay reproducibility was assessed by three tests of two whole blood samples whose CMV loads were 3.5 and 4.3 $\log_{10}$ copies/ml in three separate runs.[8]

3.1.3. Samples used to compare the COBAS Ampliprep™ and MagNA Pure 96™

209 whole blood EDTA samples were collected during the week of January 27 to February 4, 2010 by the department of virology, CHU Toulouse, France. There were kept at +4 °C until use.

3.2. Methods

3.2.1. Nucleic acid extraction

- COBAS Ampliprep Total Nucleic Acid Isolation kit® (TNAI) running on the COBAS Ampliprep™ (input/output volume: 500/75 μl, for a 6.67-fold concentration).[9]
- MagNA Pure 96 DNA and Viral NA Small Volume Kit® running on the MagNA Pure 96™ (input/output volume: 200/100 μl, for a 2-fold concentration).
- MagNA Pure LC Isolation Kit® running on the MagNA Pure LC™ (input/output volume: 200/100 μl).

3.2.2. PCR

- CMV DNA was quantified on the Light Cycler 480™.[10]
- EBV (Light Cycler EBV Quantification Kit R-GENE® (Argène, Varilhes, France),[11] BKV[12] and JCV[9] were quantified on the Light cycler 2.0™.
- The limit of detection was 500 copies (2.7 $\log_{10}$ copies/ml) for CMV, BKV and JCV and was 200 copies/ml (2.2 $\log_{10}$ copies/ml) for EBV.

3.3. Statistical analysis

StatView 5.0 was used for all statistical analyses.
Statistical significance was set at $p < 0.05$.

4. Results

4.1. Comparison of the MagNA Pure LC™, the COBAS Ampliprep™ and the MagNA Pure 96™

The CMV DNA in ten-fold dilutions of the IQC gave similar results after extraction with the three extraction systems.

Virus loads were similar for both 10^{-2} dilutions tested after extraction with the COBAS Ampliprep™ and the MagNA Pure 96™ (3 $\log_{10}$ copies/ml) and were higher than those obtained with the MagNA Pure LC™ extraction system. Only every other 10^{-2} duplicate was positive after extraction with this instrument.

CMV DNA was not detected in 10^{-3} dilutions of the IQCs regardless of the extraction system used (Table 1).

4.2. Performance of the MagNA Pure 96™ by analysis of CMV DNA

4.2.1. Sensitivity

Low concentrations of the CMV positive sample from QCMD (211 copies/ml i.e. 2.32 $\log_{10}$ copies/ml) were detected in 5/5 cases with the MagNA Pure 96™ instrument. Mean virus load was 610 copies/ml (2.78 $\log_{10}$ copies/ml; 326–890 copies/ml; 2.51–2.95 $\log_{10}$ copies/ml) indicating that the limit of detection was 500 copies/ml (2.7 $\log_{10}$ copies/ml).

4.2.2. Specificity and cross-contaminations

The specificity assessed on CMV-negative samples was 100%. Samples positive for either HSV or EBV were negative after extraction on the COBAS Ampliprep instrument™.

Extractions of alternating highly positive and negative samples showed no cross-contaminations.

4.2.3. Linearity

Linear regression analysis of the CMV data plotted against the expected concentrations yielded a regression coefficient of $R^2 = 0.998$. Linearity was good from 3.0 to 6.0 $\log_{10}$ copies/ml.

4.2.4. Intra-assay reproducibility

Seven QCMD samples with CMV loads of 3–7 $\log_{10}$ copies/ml were extracted three times in the same run. The SDs of $\log_{10}$ copies/ml varied from 0.060 to 0.28 (data not shown).

4.2.5. Inter-assay variation

The inter-assay variation was tested between two clinical samples tested three times in three consecutive runs (data not shown).

4.3. Comparison of the COBAS Ampliprep™ and the MagNA Pure 96™

- Cytomegalovirus DNA