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Performance evaluation of the automated NucliSens easyMAG nucleic acid extraction platform in comparison with QIAamp Mini kit from clinical specimens

Perandin Francesca[a,*], Pollara C. Patrizia[b], Gargiulo Franco[b], Bonfanti Carlo[a], Nino Manca[a]

[a]*Department of Experimental and Applied Medicine, Section of Microbiology, University of Brescia*
[b]*UO Microbiology, Spedali Civili, P.le Spedali Civili, 25123 Brescia, Italy*

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Abstract

The performance of the NucliSens easyMAG platform for the extraction of nucleic acid from different clinical specimens was compared with a manual procedure. A total of 308 specimens were analyzed: 209 plasma samples collected for virus detection and quantification of cytomegalovirus (CMV) and Epstein–Barr virus (EBV) ($n = 70$), and 29 for HIV genotyping for drug resistance. Linearity of extraction was tested on dilution series of CMV and EBV; the correlation coefficient (R^2) for standard curves based on repeated extraction runs was 0.99 for CMV and EBV. Inter- and intrarun variability was in accordance with previous studies, and the correlation between automated and manual extraction was very high. The concordant results were 95.7% for CMV and 100% for EBV. The results of sequence analysis for HIV drug resistance showed a concordance in 24 of 29 specimens. The NucliSens easyMAG is extremely easy to perform, is automated, and resulted in a strong reduction of hands-on time compared with manual protocol.
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Keywords: CMV; EBV; HIV; Automated extraction; Realtime PCR

1. Introduction

A large number of nucleic acid amplification tests are performed daily in an increasing number of clinical laboratories because of their high sensitivity and specificity. Advances in these techniques, including implementation of real-time polymerase chain reaction (PCR), have significantly shortened test turnaround time and hands-on time. Extraction procedures have now become the most critical and labor-intensive step in the amplification process for diagnostic assays. Because both pathogen range and the number of different sample types are expanding, there is a need for a generic extraction method (Candotti et al., 2004; Elnifro et al., 2000; Kkanna et al., 2005). Conventional manual methods are labor intensive and susceptible to handling variations, and a universal kit applicable to every type of specimen, pathogen, or nucleic acid recovery does not exist (Clewly, 1989). Recently, several new commercial systems have become attractive because their flexibility, convenience, automation, and ease of use (Tang et al., 2005).

The NucliSens easyMAG platform (bioMérieux, Boxtel, the Netherlands) is a benchtop instrument based on silica extraction technology (Boom et al., 1990). The extraction method is automated, universal, and can be applied to a broad range of different specimens such as blood, plasma, serum, sputum, and swabs; furthermore, it can be used in combination with different amplification methods.

In this study, we evaluated the performance of the NucliSens easyMAG platform for the extraction of nucleic acid from different clinical specimens, in comparison with Qiagen extraction kit (Qiagen, Hilden, Germany), and the possibility of using it in combination with different amplification systems, such as real-time PCR and sequencing.

* Corresponding author. Department of Applied and Experimental Medicine, Microbiology Department, University of Brescia, 25123 Brescia, Italy. Tel.: +39-030-3995859; fax: +39-030-395258.
E-mail address: perandin@med.unibs.it (P. Francesca).

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2. Materials and methods

2.1. Clinical specimens

A total of 308 specimens submitted to the Operative Unit of Microbiology Laboratory of Spedali Civili of Brescia, Italy, were included in this study. All were plasma samples collected to investigate the presence and obtain quantification of cytomegalovirus (CMV) (n = 209)and Epstein–Barr virus (EBV) (n = 70), 29 for HIV genotyping for drug resistance.

2.2. DNA and RNA extraction by QIAamp DNA Mini kit

DNA for CMV and EBV assay was extracted by QIAamp DNA Mini kit (Qiagen) using the "blood and body fluid protocol" as recommended in the manufacturer's instructions. CMV and EBV DNA was extracted starting from 200 μL of plasma and eluted in a final volume of 50 μL. As a control for PCR inhibitors and amplification quality, 3 μL of β-globin DNA (CPE-DNA; Nanogen Advanced Diagnostics, Turin, Italy) was added to each sample before extraction, as suggested by amplification protocol, and we amplified with β-globin primers in the same reaction mix as CMV and EBV in multiplex PCR.

For HIV drug resistance assay, peripheral blood was drawn by venipuncture into EDTA tubes, kept at room temperature, and centrifuged within 4 h of drawing at 1000 × g for 15 min. One milliliter of plasma was stored at −80 °C and thawed in a 10 to 15 °C water bath for 15 min before use. Samples were centrifuged at 23 500 × g for 75 min at 4 °C in a 2-mL microtube, and 800 μL of supernatant was removed. Manual extraction of 140 μL of original, diluted, or diluted–centrifuged samples was performed using the materials and Mini Spin protocol provided by the QIAamp viral RNA Mini kit. The RNA was eluted in a final volume of 50 μL.

Table 1
Correlation between expected results and automated extraction method performed for CMV and EBV DNA load

Expected results	CMV DNA load		EBV DNA load	
Log copies	Log copies	Ct mean	Log copies	Ct mean
2	2.24	38.12	2.74	34.61
3	3.48	33.81	3.70	31.38
4	4.43	30.50	4.73	27.96
5	5.49	26.81	5.47	25.47

The table lists Ct values and log copies.
A scatter plot of automated extraction method for CMV and EBV results reported in log copies/reaction and their correlation with the expected results.

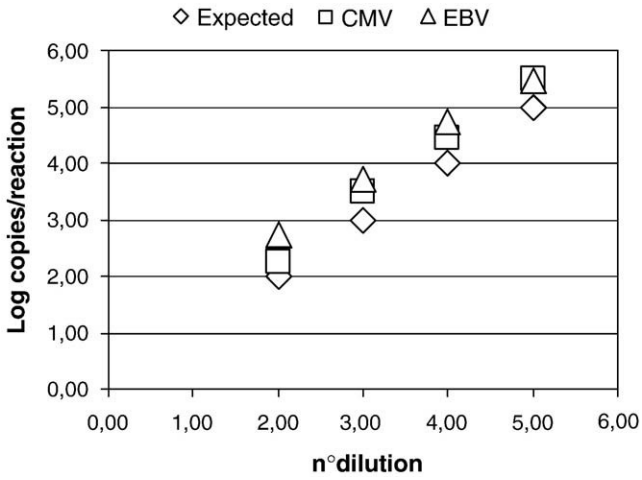

Table 2
Intra- and interassay variability of automated extraction method tested on 3 dilutions of CMV and EBV

Sample size	Result (log copies/mL)				
	Mean	Intrarun SD	Interrun SD	Total SD	Total CV (%)
2.90 log CMV (copies/mL)	2.25	0.11	0.10	0.15	6.66
3.96 log CMV (copies/mL)	3.10	0.10	0.12	0.16	5.16
4.79 log CMV (copies/mL)	3.70	0.10	0.20	0.22	5.94
2.70 log EBV (copies/mL)	2.36	0.13	0.35	0.37	15.68
3.40 log EBV (copies/mL)	3.45	0.10	0.10	0.14	4.06
4.00 log EBV (copies/mL)	3.94	0.07	0.11	0.13	3.30

CV = coefficient of variation.

2.3. NucliSens easyMAG

From a 2nd aliquot of the 308 specimens, CMV, EBV DNA, and HIV RNA were extracted by the NucliSens easyMAG platform with NucliSens magnetic extraction reagents (bioMérieux) by the onboard protocol, according to manufacturer's instructions. As a control for PCR inhibitors and amplification quality for CMV and EBV assay, 3 μL of β-globin DNA was added to each sample. Starting and final elution volume was the same as with the manual extraction system.

2.4. Viral load determination

Q-CMV PCR for the major immediate early antigen (MIEA) gene and Q-EBV PCR for a highly conserved region of EBNA-1 gene were performed in 25 μL on 5-μL DNA, according to manufacturer's instructions, using AmpliMASTER, Amplimix containing the primer set, and AmpliPROBE (Nanogen Advanced Diagnostics). DNA was quantified by AmpliSTANDARD, consisting of serial dilutions of a plasmid containing the target amplification region as multiple tubes of prediluted plasmid, ranging in concentration from 1.0×10^5 to 1.0×10^2 copies/reaction, as determined by the manufacturer's spectrophotometric reading. As reported in the manufacturer's package insert, the lower detection limit is 10 copies/mL and the dynamic range is 316 to 2.5×10^6 copies/mL. The reactions were carried out on an ABI PRISM 7300 Analytical PCR System (Applied Biosystems, Foster City, CA), according to manufacturer's instructions. The PCR cycle protocol consists of 2 min at 50 °C, 10 min at 95 °C, and 45 two-step cycles of 15 s each at 95 °C and of 1 min at 60 °C. This method allows linear quantification of 10^1 to 10^6 DNA copies per reaction, as stated by the manufacturer. To establish the concentration of

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