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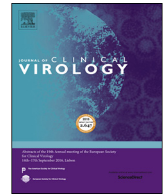

Evaluation of a new extraction platform in combination with molecular assays useful for monitoring immunosuppressed patients

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ABSTRACT

Background: In the immunosuppressed, detection of viral reactivation at the earliest convenience and molecular monitoring are of paramount importance. Nucleic acid extraction has a major impact on the reliability of results obtained from molecular assays.

Objectives: The aim of this study was to investigate the accuracy of the new EMAG® nucleic acid extraction platform and to compare the performance of the new platform to that of the standard NucliSENS® easyMAG® instrument in the routine clinical laboratory.

Study design: For accuracy testing, reference material and for comparison studies, clinical specimens were used. In addition, a lab-flow analysis including estimation of hands-on time and that for automated extraction was performed.

Results: When accuracy was tested, all 89 results obtained were found to be concordant with the results expected. When 648 clinical results were compared, 85.7% were found to be within ± 0.5 $\log_{10}$ unit, 9.5% between ± 0.5 and ± 1.0 $\log_{10}$ unit, and 4.8% more than ± 1.0 $\log_{10}$ unit. The overall time required for nucleic acid extraction of 8 samples in parallel was 94 min for the fully automated extraction mode and 82 min for the partly automated mode with the new platform, and 73 min with the standard instrument. Hands-on time was found to be shorter with the new platform.

Conclusions: The extraction performance of both platforms was found to be similar for EDTA whole blood, BAL, and urine specimens. The total turn-around time for nucleic acid extraction was found to be longer with the EMAG® platform, whereas hands-on time was reduced.

1. Background

Immune suppressive therapy after transplantation makes transplant recipients susceptible to a broad range of viral pathogens [1–6]. Multiple factors can lead to viral reactivation after transplantation, including immune suppressive therapy, graft rejection, inflammation and tissue injury [1,7–11]. The first line therapeutic decision is whether and how to reduce the intensity of immune suppression, because the risk of this therapy is graft rejection [1,12]. Several viruses including adenoviruses, cytomegalovirus (CMV), Epstein-Barr virus (EBV), enteroviruses, herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2), human herpesvirus 6 (HHV-6), influenza viruses A/B, parvovirus B19, polyomavirus BK (BKPyV), and varicella zoster virus (VZV) have been recognized as significant pathogens in immunosuppressed patients including bone marrow and solid organ transplant recipients [13,14]. In these patients, detection of reactivation at the earliest convenience and monitoring of viral disease and antiviral treatment through molecular monitoring is of paramount importance [15]. Today, real-time PCR (qPCR)-based techniques are the method of choice for detection and quantitation of viruses in immunosuppressed patients. For the routine diagnostic laboratory, maximum automation of molecular assays is of major importance [13]. Several automated nucleic acid extraction platforms have been brought on the market and performance studies have been done [16–23]. Through introduction of automation, manual work can be reduced and reliability of results increased [19].

The newly introduced fully-automated EMAG® platform (bioMérieux S.A., Marcy l´Etoile, France) allows parallel extraction of DNA's and RNA's from different sample materials. In contrast, the NucliSENS® easyMAG® (bioMérieux) instrument requires manual addition of clinical specimens, internal controls, and magnetic silica particles. While the NucliSENS® easyMAG® allows parallel extraction of 24 specimens, the new EMAG® is able to perform a maximum of 48 extractions in a fully automated mode.

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2. Objectives

The aim of this study was to compare the performance of the new EMAG® platform and the NucliSENS® easyMAG® instrument. For experiments, commercially available reference material and clinical samples obtained from patients with immunosuppression were used. Additionally, turn-around and hands-on times were compared.

3. Study design

All experiments were done in an International Standard Organization (ISO 9001:2008)-certified laboratory, the Molecular Diagnostics Laboratory at the Medical University of Graz. Throughout the whole study, all tests were performed according to the manufacturer's package insert instructions.

For accuracy testing, adenovirus, CMV, EBV, enterovirus, HSV-1, HSV-2, HHV-6, influenza virus A/B, parvovirus B19, BKPyV, and VZV panels from the Quality Control for Molecular Diagnostics (QCMD; https://www.qcmd.org/) 2015 and 2016 panels were used. Additionally, the 1 st WHO International Standard for Cytomegalovirus and the 1 st WHO International Standard for Epstein-Barr virus provided by the National Institute for Biological Standards and Controls (http://www.nibsc.org/) were employed. Dilutions of 1:10 and 1:100 were prepared using PCR-grade water (Roche Diagnostics, Penzberg, Germany) according to the instructions in the package insert. All experiments were performed using the EMAG® platform for nucleic acid extraction.

For the clinical study, 63 anonymized left-over specimens that had been obtained from female and male patients with immunosuppression and suspected viral reactivation treated at different departments of the University Hospital Graz were studied. Blood specimens had been collected in 9-mL K-EDTA tubes (Greiner Bio-One, Kremsmünster, Austria), BAL fluid specimens in 12-mL sterile screw cap tubes (Greiner Bio-One), and urine specimens in 10-mL VACUETTE® Urine CCM tubes (Greiner Bio-One) and transferred to the Molecular Diagnostics Laboratory for routine testing. The remaining sample materials were coded and studied anonymized. Pathogens and corresponding sample materials are shown in Table 1. One-mL aliquots containing EDTA whole blood and 1.5-mL aliquots containing BAL fluids or urine were prepared in FALCON® tubes (Corning Science México S.A. de C.V., Tamaulipas, México) and frozen at −70 °C until further use. After thawing, samples were processed in parallel throughout the whole study; i.e., sample aliquots for the extraction platforms were always pipetted out of a single tube and qPCR mixes including eluates from either the EMAG® platform or the NucliSENS® easyMAG® instrument were always amplified and detected on the identical multiwell plate.

Table 1
Pathogens tested in 63 anonymized left-over specimens.

Pathogen	Type of specimen		
	EDTA whole blood (n = 23)	Urine (n = 20)	BAL fluid (n = 20)
Adenoviruses	X	X	X
CMV	X	X	
Enteroviruses			X
EBV	X		
HSV-1/HSV-2	X		
HHV-6	X		
Influenza A/B			X
Parvovirus B19	X		
BKPyV		X	
VZV	X		

BAL, bronchoalveolar lavage; CMV, cytomegalovirus; EBV, Epstein-Barr virus; HSV-1, herpes virus type 1; HSV-2, herpes virus type 2; HHV-6, human herpes virus 6; BKPyV, polyomavirus BK; VZV, varicella zoster virus.

3.1. Nucleic acid extraction

For accuracy testing, reference material was extracted with the EMAG® platform, together with negative and positive controls included in each R-GENE® (ARGENE®, bioMérieux) amplification and detection kit. Only panel members with expected results within the analytical measuring range of the specific test employed and negatives were tested. Additionally, the internal control (IC) for exclusion of possible inhibition was added.

For the clinical study, different sample materials obtained from a single patient were extracted in a single run on both platforms in parallel. Prior to the start of the nucleic acid extraction procedure, test requests were entered and transferred to ARGENE® CONNECT, a data management software with a bidirectional interface with the laboratory information system (LIS), where the nucleic acid extraction protocols were defined for each sample. Immediately after thawing, 200 µl of EDTA whole blood and 500 µl of urine or BAL fluid were pipetted into the extraction vessel of the NucliSENS® easyMAG® instrument. Thereafter, FALCON® tubes containing blood or urine were placed directly into the sample rack of the EMAG® platform, while those containing BAL fluid were pipetted manually into the vessels.

After completion of the automated extraction procedure, eluates including DNA´s and RNA´s are provided in vessels. After assessment of each nucleic acid extraction run, the identity of each sample and the specific test request(s) were transferred to ARGENE® CONNECT for qPCR setup on the ESTREAM® (bioMérieux) platform.

3.2. qPCR setup

Vessels containing eluates were transferred to the ESTREAM® platform. This platform automatically pipets qPCR mixes and eluates into the wells of multiwell plates placed on a cooling unit. According to the information received from ARGENE® CONNECT, the ESTREAM® platform performs the qPCR setup automatically. In addition to the test requests, special qPCR mixes containing a cellular control (CC) checking for presence of human cells in the samples are prepared for eluates obtained from BAL fluids.

3.3. Amplification and detection

Amplification and detection was performed with commercially available in vitro diagnostics (IVD)/Conformité Européenne (CE)-labeled molecular tests. Performance data of these tests are shown in Table 2.

After completion of the qPCR setup, the multiwell plate was transferred to the LightCycler® 480 II (Roche Molecular Diagnostics, Rotkreuz, Switzerland). In parallel, the qPCR setup file of the multiwell plate was transferred from the ESTREAM® to the LightCycler® 480 II, allowing assignment of each sample identity to the result obtained. For amplification and detection on the LightCycler® 480 II instrument, the Respiratory R-GENE® amplification program was chosen. This program allows parallel amplification of DNA´s and RNA´s without the need of separation of DNA and RNA viruses. All samples were thus processed under identical conditions.

3.4. Reporting results

The viral target was analyzed at 530 nm (FAM), the IC at 560 nm (HEX). If the difference between the crossing point (CP) value obtained from a single IC and that obtained from the negative control was less than three cycles, the results was assigned as “positive” or “target not detected”. If the difference between the CP values exceeded three cycles, the result was assigned as inhibited.

Quantitative results were generated by using the Absolute Quantification mode. Prior to the first run of the study, a calibration curve using the four quantitation standards provided with each

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