JCM
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Performance of the MagNA Pure 96 System for Cytomegalovirus Nucleic Acid Amplification Testing in Clinical Samples

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This report shows the performance of MagNA Pure 96 automated nucleic acid extraction for the quantitative detection of cytomegalovirus DNA in clinical samples by real-time PCR. The obtained results demonstrate that this workflow is characterized by high sensitivity and linearity and ensures reliable, reproducible clinical diagnostics.

A critical feature for reliability of results in routine molecular diagnostics is the extraction of target DNA and RNA. The Roche MagNA Pure 96 system was launched in 2009 for automated nucleic acid extraction applicable in life science research. To use this automated platform also for *in vitro* diagnostics, it has to be shown that the performance of the MagNA Pure 96 system complies with the essential requirements of European Directive 98/79/EC on *in vitro* diagnostic medical devices (1). Accordingly, CE marking on the MagNA Pure 96 system declares the capability of the product to extract pure nucleic acids with high recovery and reproducibility, which ensures reliable clinical diagnostics. Evaluation of MagNA Pure 96 nucleic acid extraction using clinical respiratory specimens for identifying viruses by qualitative real-time PCR was recently reported (2). Rapid, sensitive, accurate, and predictive molecular cytomegalovirus (CMV) assays in the routine diagnostic laboratory are of increasing interest for the prevention and management of CMV diseases in immunocompromised patients, such as transplant recipients (3, 4). In addition, CMV screening is of particular importance concerning pregnancy, because CMV is one of the most common causes of congenital infections (5, 6).

The present study evaluated the performance of the MagNA Pure 96 system for CMV DNA isolation from 200 μl of cell culture (standard material) and of clinical plasma specimens. The extraction on the MagNA Pure 96 system was carried out with the "Viral NA Plasma Protocol" and/or with the "Pathogen Universal Protocol" (elution volume, 50 μl). In comparison, the "Cell-free Protocol" on the QIAsymphony SP platform (Qiagen, Hilden, Germany) was used for sample processing (elution volume, 85 μl) in our in-house CMV workflow. Data of studies that have evaluated the QIAsymphony SP extraction platform in diagnostic laboratories were previously published (7–9).

Isolated CMV DNA was quantified using a real-time PCR assay according to Mori et al. (10) by applying a two-step protocol on a Roche LightCycler 2.0 instrument. All samples were processed together with 100 copies of a plasmid-based internal control of lambda DNA flanked by CMV primer sequences to exclude false-negative results.

At the beginning of the study, the viral load of our CMV standard material was verified on a Cobas Amplicor Analyzer using the Cobas Amplicor CMV Monitor test (Roche Diagnostics). Then, dilutions of the standard material were subjected to multiple processing in one run and in runs on several days according to the recommendations of the European Pharmacopoeia to determine performance parameters. The obtained low intra- and inter-run variations demonstrated a good recovery of the viral loads tested (coefficient of variation, ≤2%; standard deviation, less than ±0.5 copies/ml). The limit of detection (LOD), the point at which 95% of the replicates of a given viral load are detected, was determined by a statistical approach (Probit analysis). The LOD of the CMV workflow that included MagNA Pure 96 extraction with the Viral NA Plasma Protocol as well as with the Pathogen Universal Protocol was found to be 330 copies/ml. This result represents an analytical sensitivity slightly higher than that determined for our routine CMV workflow with the QIAsymphony SP (LOD = 750 copies/ml). Excellent linearity of quantitative CMV testing was observed at all times (Pearson's correlation coefficient $r > 0.999$). For each CMV workflow tested (MagNA Pure 96 extraction with the Viral NA Plasma Protocol as well as with the Pathogen Universal Protocol; QIAsymphony SP extraction with the Cell-free Protocol), a linear measurement range between 1,000 copies/ml

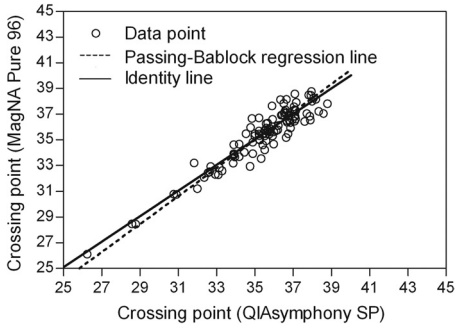

FIG 1 Correlation between CMV workflows including the MagNA Pure 96 system and the QIAsymphony SP platform. A line-fitted plot of regression analysis comparing CMV real-time PCR results via crossing points after DNA extraction on the MagNA Pure 96 system and on the QIAsymphony SP platform ($r = 0.9466$) is shown.

Received 13 December 2012 **Returned for modification** 10 January 2013
Accepted 25 February 2013
Published ahead of print 6 March 2013
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doi:10.1128/JCM.03289-12

TABLE 1 Particular features of QIAsymphony SP and MagNA Pure 96

Feature	Characteristic[a]	
	QIAsymphony SP	MagNA Pure 96
Extraction kit	Varies as a function of sample material and vol	Varies as a function of sample vol
Extraction protocol	Varies as a function of sample material	One for all materials
Capacity	1–24 samples in parallel	1–96 samples in parallel (batch of 8)
Rapidity (96 samples)	4 h	1 h
Use of primary test tubes	+	−
Scanning of primary test tubes	+	−
Sample handling	QIAsymphony SP pipetting	Manual pipetting
Dead vol	150 μl	
Elution tube(s)	Variable	96-well plate
Eluate handling	Manual pipetting	MagNA Pure 96 pipetting into 96-well plate (1:1)
Maintenance	4 O-ring changes (monthly)	

[a] +, yes; −, no.

and 3,000,000 copies/ml was determined. A recovery rate of 25% (admissible deviation from the viral load recovered through CMV workflow procedures expressed as a percentage) was used as a criterion of linearity (including Passing-Bablok regression).

In addition, 998 consecutive clinical plasma specimens were tested for CMV DNA after processing in parallel on the QIAsymphony SP platform and on the MagNA Pure 96 system using the Pathogen Universal Protocol was performed. High concordance between the results of the two CMV workflows was found as shown in Fig. 1 on the basis of crossing points generated for DNA amplification. In agreement, 109 samples tested positive for CMV, whereas no CMV DNA was detected in 861 samples. In addition, 26 samples were found positive only after extraction on the MagNA Pure 96 system, probably due to the slightly lower LOD of the corresponding workflow. However, viral DNA below the minimum possible value for the linear measurement range was detected exclusively in two samples when the routine workflow with extraction on QIAsymphony SP platform was applied. This minor variance is probable due to the definition of LOD as the point at which 95% of the critical viral load is detected. Using statistical methods for assessing agreement between the two CMV workflows, sensitivity and specificity of more than 97% for the MagNA Pure 96 system applying the Pathogen Universal Protocol were calculated. To compare the two extraction systems in more detail, particular differences between and features of the QIAsymphony SP platform and the MagNA Pure 96 system are summarized in Table 1.

In summary, the results of the present study reveal high sensitivity, precision, and reproducibility of the CMV workflow including the MagNA Pure 96 system. In addition, the robustness of the assay is exposed by small intra- and interrun variations, which are the result of the ability of the clinical test to withstand small changes in method parameters. Our results confirm the usability of the MagNA Pure 96 system, especially for high-throughput extraction of CMV DNA from clinical plasma samples, to ensure reliable, reproducible quantitative diagnostics.

REFERENCES

1. **European Parliament, Council of 27 October 1998.** 12 December 1998. Directive 98/79/EC on in vitro diagnostic medical devices. Off. J. Eur. Community **L331**:1–37. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31998L0079:en:HTML.
2. **Mengelle C, Mansuy JM, Sandres-Sauné K, Barthe C, Boineau J, Izopet J.** 2012. Prospective evaluation of a new automated nucleic acid extraction system using routine clinical respiratory specimens. J. Med. Virol. **84**:906–911.
3. **Scott GM, Naing Z, Pavlovic J, Iwasenko JM, Angus P, Jones R, Rawlinson WD.** 2011. Viral factors influencing the outcome of human cytomegalovirus infection in liver transplant recipients. J. Clin. Virol. **51**:229–233.
4. **Kotton CN.** 2010. Management of cytomegalovirus infection in solid organ transplantation. Nat. Rev. Nephrol. **6**:711–721.
5. **Berger A, Reitter A, Harter PN, Buxmann H, Allwinn R, Louwen F, Doerr HW.** 2011. Problems and challenges in the diagnosis of vertical infection with human cytomegalovirus (CMV): lessons from two accidental cases. J. Clin. Virol. **51**:285–288.
6. **Yinon Y, Farine D, Yudin MH, Gagnon R, Hudon L, Basso M, Bos H, Delisle MF, Menticoglou S, Mundle W, Ouellet A, Pressey T, Roggensack A, Boucher M, Castillo E, Gruslin A, Money DM, Murphy K, Ogilvie G, Paquet C, Van Eyk N, van Schalkwyk J.** 2010. Cytomegalovirus infection in pregnancy. J. Obstet. Gynaecol. Can. **32**:348–354.
7. **Parham NJ, Parmar SA, Kumar N, Aliyu S, Curran MD, Zhang H.** 2012. Automated nucleic acid isolation in viral molecular diagnostics: evaluation of the QIAsymphony SP. Br. J. Biomed. Sci. **69**:18–25.
8. **Verheyen J, Kaiser R, Bozic M, Timmen-Wego M, Maier BK, Kessler HH.** 2012. Extraction of viral nucleic acids: comparison of five automated nucleic acid extraction platforms. J. Clin. Virol. **54**:255–259.
9. **Lee AV, Atkinson C, Manuel RJ, Clark DA.** 2011. Comparative evaluation of the QIAGEN QIAsymphony SP system and bioMérieux NucliSens easyMAG automated extraction platforms in a clinical virology laboratory. J. Clin. Virol. **52**:339–343.
10. **Mori T, Okamoto S, Matsuoka S, Yajima T, Wakui M, Watanabe R, Ishida A, Iwao Y, Mukai M, Hibi T, Ikeda Y.** 2000. Risk-adapted pre-emptive therapy for cytomegalovirus disease in patients undergoing allogeneic bone marrow transplantation. Bone Marrow Transplant. **25**:765–769.