JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2007, p. 421–425 Vol. 45, No. 2
0095-1137/07/$08.00+0 doi:10.1128/JCM.00894-06

Evaluation of NucliSens easyMAG for Automated Nucleic Acid Extraction from Various Clinical Specimens[▽]

K. Loens,* K. Bergs, D. Ursi, H. Goossens, and M. Ieven

Department of Microbiology, University of Antwerp UIA, Antwerp, Belgium

Received 28 April 2006/Returned for modification 22 June 2006/Accepted 5 December 2006

The objectives of this study were to evaluate the performance of the NucliSens easyMAG platform for nucleic acid extraction from different clinical specimens compared to NucliSens miniMAG platform and manual QIAGEN extraction. The NucliSens easyMAG and the NucliSens miniMAG showed equal performance on 215 throat swabs since real-time nucleic acid sequence-based amplification scored the same samples positive for *Mycoplasma pneumoniae* ($n = 9$) and *Chlamydia pneumoniae* ($n = 5$) RNAs, although internal control RNA was slightly better detected with the NucliSens easyMAG (99.3% versus 96.8%). NucliSens easyMAG extracted nucleic acids more efficiently (higher recovery and/or fewer inhibitors) compared to QIAGEN extraction by showing, on average, lower Ct values in real-time LightCycler PCR, although 4 individual specimen out of 45 were found positive only with QIAGEN. For nine *M. pneumoniae*-positive throat swabs, the mean difference in Ct values between NucliSens easyMAG extraction and QIAGEN extraction was −2.26 (range, −5.77 to +0.60); for the detection of five *C. pneumoniae*-positive throat swabs, the average difference in Ct values between the two methods was −3.38 (range, −6.62 to −2.02); and for the detection of cytomegalovirus in 24 blood samples, the mean difference in Ct values between the two methods was −0.95 (range, −5.51 to +1.68). The NucliSens easyMAG is considerably easier to perform, efficiently extracts nucleic acids from throat swabs and whole blood, is automated, and has high throughput.

Numerous nucleic acid amplification tests are performed daily in an increasing number of clinical laboratories because of their high sensitivities and specificities. Further developments in the field have decreased the turnaround time and hands-on time. Nucleic acid extraction systems with high flexibilities in the type and number of samples to be handled and with a wide range of sample and elution volumes and short turnaround times provide a further advantage to adapt amplification techniques to clinical diagnostic requirements.

A high-quality nucleic acid extract is expected to be free of amplification inhibitors and other substances that might affect enzyme substrates, and the target should be optimally recovered.

The NucliSens easyMAG platform (bioMérieux, Boxtel, The Netherlands) is a second-generation system for automated isolation of nucleic acids from clinical samples based upon silica extraction technology (3). It is a benchtop instrument with the same reagents as the manual version, i.e., NucliSens miniMAG platform (bioMérieux) (4, 14, 17, 19). Manual steps are limited to the loading of samples, reagents, and disposables. One to 24 samples can be analyzed in one run. The extraction method is universal and can be applied to a broad range of different specimens such as blood, sputum, serum, and throat swabs. The instrument can be used in combination with different amplification methods such as nucleic acid sequence-based amplification (NASBA) or PCR. Limited data are available on the NucliSens easyMAG extraction procedure (19).

The objectives of this study were to evaluate the performance and user convenience of the NucliSens easyMAG platform for the extraction of nucleic acid from different clinical specimens in comparison to those of QIAGEN extraction and NucliSens miniMAG extraction.

MATERIALS AND METHODS

Patients and specimens. A total of 215 dry throat swabs from 215 patients with X-ray-proven community-acquired pneumonia were investigated. All patients were prospectively included in a hospital-based study across Europe starting in October 2002 and lasting until May 2003. All specimens were stored locally at −20°C and regularly shipped on dry ice to the central laboratory in Antwerp, Belgium. Upon arrival in the laboratory, specimens were suspended in 1 ml of sterile saline, aliquoted in portions of 100 μl, and stored at −70°C until they were processed batchwise. For extraction by the NucliSens miniMAG platform, 900 μl of lysis buffer (bioMérieux) was added to the protease-treated aliquots of 100 μl (11). The samples were mixed vigorously for rapid lysis and stored at −70°C. Aliquots used for NucliSens EasyMAG extraction were stored at −70°C for 1 year.

From 24 cytomegalovirus (CMV) DNA-positive EDTA blood specimens from transplant patients, nucleic acids were isolated after storage for up to 3 months at −20°C without the addition of lysis buffer, except for 6 EDTA blood samples which were analyzed as fresh specimens.

Nucleic acid extraction. *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* DNAs were extracted from 215 throat swabs with the QiaAmp blood mini kit (QIAGEN, Hilden, Germany) according to the instructions of the manufacturer. Elution was done in 100 μl of elution buffer. From a second aliquot of the 215 specimens, *M. pneumoniae* and *C. pneumoniae* RNAs were extracted by the NucliSens miniMAG platform with the NucliSens magnetic extraction reagents (bioMérieux, Boxtel, The Netherlands) according to the instructions of the manufacturer. Elution of these nucleic acid extracts was done in 20 μl. The third aliquot was subjected to the NucliSens easyMAG platform for total nucleic acid extraction (bioMérieux) by the off-board protocol according to the instructions of the manufacturer. Nucleic acids were eluted in 20 μl of elution buffer.

CMV DNA was extracted from 200 μl of EDTA blood with the QiaAmp blood mini kit (QIAGEN) according to the instructions of the manufacturer. Nucleic acids were eluted in 75 μl of elution buffer. A similar sample volume was retrospectively used for NucliSens easyMAG extraction by the off-board protocol. Nucleic acids from the EDTA blood were eluted in 60 μl. This volume was chosen since it was not possible to elute in 75 μl with the easyMAG.

* Corresponding author. Mailing address: Department of Medical Microbiology, University of Antwerp, Universiteitsplein 1 S009a, B-2610 Wilrijk, Belgium. Phone: 32-3-820-27-51. Fax: 32-3-820-26-63. E-mail: Katherine.loens@ua.ac.be.

▽ Published ahead of print on 13 December 2006.

TABLE 1. Overview of *M. pneumoniae*- and *C. pneumoniae*-positive throat swabs

Organism and patient no.	LightCycler Ct value after:		Result reported by NucliSens easyQ software (NASBA)	
	QIAGEN extraction	easyMAG extraction	miniMAG extraction	easyMAG extraction
M. pneumoniae				
1	33.06	29.18	Positive	Positive
2	25.85	26.45	Positive	Positive
3	36.87	31.10	Positive	Positive
4	31.16	28.33	Positive	Positive
5	29.20	28.00	Positive	Positive
6	33.96	33.50	Positive	Positive
7	36.88		Negative	Negative
8	29.34	26.87	Positive	Positive
9	31.72	29.82	Positive	Positive
10	33.25	30.76	Positive	Positive
C. pneumoniae				
11	31.37	28.59	Positive	Positive
12	33.30	30.98	Positive	Positive
13	34.65	31.51	Positive	Positive
14	36.35	34.33	Positive	Positive
15	35.72	29.10	Positive	Positive

TABLE 2. Overview of CMV-positive EDTA blood samples[a]

Patient no.	LightCycler Ct value after:	
	QIAGEN extraction	EasyMAG extraction
1	27.02	26.22
2[b]	37.93	
3	34.84	31.87
4	38.09	36.81
5	36.86	34.91
6	35.11	36.79
7	28.09	26.00
8	33.79	32.76
9[c]	38.13	
10	35.55	34.62
11	36.46	35.07
12[c]	37.71	
13	30.05	29.82
14	36.11	36.35
15	37.11	38.25
16	34.91	35.70
17	35.13	31.90
18	35.47	35.93
19	38.00	36.83
20	42.76	37.25
21	34.35	33.96
22	33.07	31.25
23	35.92	35.25
24	36.85	38.15

[a] Nucleic acids were extracted from samples 1 to 20 by the easyMAG after storage for up to 3 months at −20°C. Samples 19 to 24 were freshly collected EDTA blood samples.

[b] Result negative after re-extraction and reamplification; this patient was never found to be CMV positive before.

[c] Transplant patient known to be CMV positive.

In this study, the QIAGEN nucleic acid extraction system and the NucliSens miniMAG platform were used as reference methods for extraction of DNA and RNA, respectively.

Nucleic acid amplification assays. Nucleic acid extracts from the 215 throat swabs obtained with the NucliSens miniMAG and NucliSens easyMAG were investigated by real-time NASBA with the NucliSens EasyQ *M. pneumoniae* assay and the NucliSens EasyQ *C. pneumoniae* assay (bioMérieux) according to the instructions of the manufacturer. Both assays contain internal control RNA.

The amplification process was run in a fluorescence reader, the NucliSens EasyQ Analyzer (bioMérieux). The results obtained with the NucliSens Easy Q assay were calculated with the NucliSens EasyQ software and were classified as positive, negative, or invalid if the internal control was not detected or the signal was too weak. In negative control reaction mixtures, the target nucleic acid was replaced with RNase- and DNase-free water.

M. pneumoniae (22) and *C. pneumoniae* real-time PCRs (6) were done as described previously. For the detection of CMV, real-time PCR with the LightCycler was performed according to Stöcher (18).

Reproducibility of QIAGEN and NucliSens easyMAG extraction. The intrarun variations in extraction by both QIAGEN and NucliSens easyMAG were estimated by extracting five aliquots of the same CMV-positive EDTA blood sample within the same run by each method.

RESULTS

Throat swabs. The real-time PCR detected *M. pneumoniae* and *C. pneumoniae* in 10 and 5 throat swabs after QIAGEN extraction and in 9 and 5 swabs after easyMAG extraction, respectively. For the detection of the nine *M. pneumoniae*-positive specimens, the differences between the mean LightCycler Ct values obtained after NucliSens easyMAG extraction and QIAGEN extraction were, respectively, −3.88, 0.6, −5.77, −2.83, −1.2, −0.46, −2.47, 1.9, and −2.49 (Table 1). For the detection of five *C. pneumoniae*-positive specimens, the LightCycler Ct values obtained for the NucliSens easyMAG were, respectively, −2.78, −2.32, −3.14, −2.02, and −6.62 compared to the results obtained after QIAGEN extraction.

Real-time NASBA detected *M. pneumoniae* and *C. pneumoniae* in nine and five throat swabs after extraction by both NucliSens miniMAG and NucliSens easyMAG (Table 1). The number of invalid results in negative specimens obtained after real-time NASBA decreased from 14 (3.2%) to 3 (0.7%) with the NucliSens miniMAG and NucliSens easyMAG, respectively. The internal control RNA was detected slightly better with the NucliSens easyMAG compared to the NucliSens miniMAG (99.3 versus 96.8%, respectively).

Statistical analysis. For the throat swabs (raw data, being either positive or negative), there was one specimen which was found positive only after QIAGEN extraction and real-time PCR amplification and detection. This is not statistically significant. In addition, looking in more detail at the data (Ct values) showed improvements in Ct values after NucliSens easyMAG extraction.

EDTA blood. Real-time PCR detected CMV DNA in nucleic acids extracts from EDTA blood from 24 patients. All six fresh blood sample extracts were found to be positive after both QIAGEN and NucliSens easyMAG nucleic acid extraction. The differences between the mean LightCycler Ct values obtained after NucliSens easyMAG extraction and QIAGEN extraction were −5.5, −1.82, −1.17, −0.67, −0.39, and +1.3. For the other 18 samples stored for up to 3 months at −20°C before extraction by the NucliSens easyMAG, 15 nucleic acid extracts were found to be positive after easyMAG extraction. Two out of these three patients scored negative in combination with NucliSens easyMAG extraction were known CMV-positive transplant patients, and re-extraction with QIAGEN and reamplification confirmed the results. In contrast, the third patient (Table 2, patient 2) had never been found to be CMV

positive before and re-extraction with QIAGEN and reamplification did not confirm the positive result. An overview of these results is presented in Table 2.

Statistical analysis. For detection of CMV in blood, raw data (positive/negative) yielded two samples which were found positive after QIAGEN extraction. The Ct values were suggestive of small DNA amounts. This is not statistically significant. But again, looking closer revealed that the DNA was extracted more efficiently in 15/23 blood samples.

Reproducibility of QIAGEN and NucliSens easyMAG extraction. The QIAGEN intrarun coefficient of variation and standard deviation are 3.540 and 1.212, respectively. For the NucliSens easyMAG extraction, an intrarun coefficient of variation and standard deviation of 1.239 and 0.440, respectively, were obtained.

User convenience of the NucliSens easyMAG. The NucliSens easyMAG represents a universal highly flexible extraction instrument with which (i) different sample input and elution volumes within the same run are possible, (ii) 1 to 24 samples can be treated in one run, and (iii) DNA and RNA extraction can be performed within the same run. Furthermore, the same NucliSens easyMAG nucleic acid extract can be used for both RNA and DNA amplification. The turnaround time for DNA or RNA extraction with the NucliSens easyMAG was reduced to 40 min for 24 samples by the off-board protocol, including about 10 min of hands-on time, whereas nucleic acid extraction of 12 samples by QIAGEN takes 50 min starting from the addition of the lysis buffer. The consumable and disposable costs for QIAGEN DNA extraction, QIAGEN RNA extraction, and NucliSens easyMAG DNA or RNA extraction were $4.10, $5.00, and $7.90, respectively.

DISCUSSION

Conventional manual nucleic acid extraction for the isolation of DNA or RNA from clinical samples is the most labor-intensive and critical part of current nucleic acid amplification assays for pathogen detection. Evaluations and comparisons of different extraction methods have been performed with a variety of specimen types, target organisms, and assays (8, 9, 16, 24). These comparisons are important for determining the effectiveness of nucleic acid extraction and removal of enzymatic inhibitors, since these have a direct influence on the result of the amplification assay. The use of automated nucleic acid extraction methods has been previously shown to be an acceptable and possibly superior replacement for the use of manual methods because of the reduction in technician time (1, 8, 10, 13, 24, 25).

The purpose of this study was to evaluate the performance and user convenience of the NucliSens easyMAG platform for the isolation of RNA and/or DNA from throat swabs and EDTA blood and from various organisms. In this study, the QIAGEN nucleic acid extraction system and the NucliSens miniMAG platform were used as reference methods for DNA and RNA extraction, respectively.

Real-time PCR detected *M. pneumoniae* and *C. pneumoniae* in 10 and 5 throat swabs after QIAGEN extraction and in 9 and 5 swabs after NucliSens easyMAG extraction. For the detection of the positive specimens, the difference between the LightCycler Ct values obtained after NucliSens easyMAG extraction and QIAGEN extraction varied between −6.62 and 0.6, resulting from a higher DNA yield or from fewer inhibitors being present in the nucleic acid extract. In most cases, better results were obtained after easyMAG extraction, even when the specimen was stored for more than 1 year at −70°C in the absence of a stabilizing lysis buffer, meaning that DNA degradation did not occur frequently. It could also not be related to inter- or intrarun variations since, e.g., the mean crossing point for *M. pneumoniae* real-time PCRs for amplification of the lowest dilution was 26.64 with a standard deviation of 0.056 while the mean crossing point for amplification of the highest dilution was 33.48 with a standard deviation of 0.315. For calculation of interassay variation, the mean crossing point was 33.81 with a standard deviation of 0.457. Similar inter- and intrarun variations were obtained for *C. pneumoniae* real-time PCR. One specimen was repeatedly *M. pneumoniae* positive after QIAGEN extraction but negative after NucliSens easyMAG extraction. The LightCycler Ct value suggests that the sample contained very small amounts of DNA, which could have been degraded after 1 year of storage at −70°C in the absence of a nucleic acid stabilizing buffer.

Lower LightCycler Ct values were obtained in 14/21 (66%) of the NucliSens easyMAG nucleic acid extracts from the EDTA CMV-positive blood samples, again suggesting that a higher DNA yield or a cleaner nucleic acid extract could be obtained after NucliSens easyMAG extraction. Three discordant results were obtained (patients 2, 9, and 12), all in cases where the LightCycler Ct values indicated that small amounts of CMV DNA were present in the sample. Two of these transplant patients were known to be CMV positive; one patient had never been found to be CMV positive before. After re-extraction in duplicate with QIAGEN and reamplification, negative results were obtained for the latter. The positive result was probably due to carryover contamination. The EDTA specimens from the other two patients were selected on the basis of PCR positivity for CMV and retrospectively analyzed by the NucliSens easyMAG. The negative results might be due to DNA degradation.

Other data from the literature indicate also that the sensitivity of a nucleic acid amplification assay after nucleic acid extraction with an automated system is similar to or better than the sensitivity obtained after manual nucleic acid extraction. When Wilson et al. (24) compared five commercially available methods for extraction of *Legionella pneumophila* from respiratory specimens, higher concentrations of *L. pneumophila* DNA were recovered from sputum with the MagNa Pure LC (Roche Diagnostics) and the NucliSens extractor (bioMérieux) than by the manual methods, i.e., the High Pure PCR Template Preparation kit (Roche Diagnostics), the QiaAmp DNA Mini kit (QIAGEN), and the ViralXpress kit (Chemicon). Exner and Lewinski compared the MagNa Pure system with the QiaAmp DNA blood mini kit and with phenol-chloroform extraction for the detection of *Borrelia burgdorferi* DNA from various types of specimens (5). Comparable sensitivities for all of the methodologies were obtained with all specimen types except urine, in which case QiaAmp extraction was two times less sensitive. On the other hand, Schuurman et al. (15) mentioned that MagNa Pure extraction of spiked CMV in whole blood showed a 5- to 10-fold reduction in PCR sensitivity compared to the manual nucleic acid extraction according to

Boom et al. (3). On the basis of our present knowledge, it would be of interest to compare the NucliSens easyMAG platform with other automated nucleic acid extractors.

NucliSens easyMAG extraction is an improvement over NucliSens miniMAG extraction in terms of user friendliness, whereas both methods are comparable in extraction efficiency in terms of RNA recovery.

Similar findings were reported by Tang et al. (19), who validated the NucliSens easyMAG by using 75 urine specimens for detection of the polyomavirus BK virus. Its sensitivity for detection of the BK virus was identical to that of the miniMAG, the MagNa Pure compact system, and the BioRobot EZ1. Its reproducibility was similar to that of the NucliSens miniMAG, and the human β-actin gene was detected in 71 (94.7%) of the urine specimens.

The type of nucleic acid amplification test inhibitors present and the composition of the clinical specimen vary depending on the type of infecting organism and the site from which the clinical specimen was obtained. Since throat swabs represent the majority of respiratory specimens for detection of *M. pneumoniae* and *C. pneumoniae* in our laboratory and whole blood is used for CMV detection by nucleic acid amplification tests, the efficiency of nucleic acid extraction for *M. pneumoniae*, *C. pneumoniae*, and CMV in throat swabs and whole blood, respectively, was studied.

In the literature, inhibition of amplification in respiratory specimens varies between 0 and 25%, depending on the type of specimens used (2, 7, 20, 21, 23). The use of a sample integrity control such as U1A (12) or an internal control added to each sample could exclude false-negative results due to inhibitors present in respiratory specimens. The internal control included in the NucliSens EasyQ *M. pneumoniae* assay and the NucliSens EasyQ *C. pneumoniae* assay provides information about the performance of the extraction, amplification, and detection procedures and the quality of the reagents in each particular run. Without the internal control, 3.2 and 0.7% would have been reported negative by the NucliSens miniMAG and easyMAG, respectively. No invalid results were obtained after real-time PCR applied on the NucliSens easyMAG nucleic acid extract.

A major concern in the use of automated nucleic acid extraction is the risk of cross contamination of negative specimens by strongly positive specimens as a consequence of aerosols, leaking pipettes, or faulty robotics. No false-positive results were obtained after NucliSens easyMAG extraction, whereas one false-positive CMV result was suspected after QIAGEN extraction. These samples are a known risk in our laboratory for cross contamination during the numerous pipetting and centrifugation steps inherent in the manual extraction procedure. Manual extraction therefore requires more rigorous handling and expertise from the analyst. However, no carryover study was performed.

In this study, the NucliSens easyMAG extracted DNA and RNA more efficiently from clinical samples (higher recovery and/or fewer inhibitors) than did QIAGEN extraction by showing, on average, lower Ct values in the LightCycler real-time PCR assay. The instrument features user-friendly intuitive software; allows nucleic acid extraction from different types of specimens with different input and elution volumes, as well as DNA and RNA targets, in a single extraction run; and delivers high throughput capabilities with a 40-min turnaround time. The ability to extract the majority of samples automatically with such a generic extraction protocol can lead to a large reduction in the total turnaround time, especially since laboratories often use different manual sample preparation protocols and kits for DNA and RNA targets.

REFERENCES

1. **Beuselinck, K., M. Van Ranst, and J. Van Eldere.** 2005. Automated extraction of viral pathogen RNA and DNA for high-throughput quantitative real-time PCR. J. Clin. Microbiol. **43:**5541–5546.
2. **Blackmore, T. K., M. Reznikow, and D. L. Gordon.** 1995. Clinical utility of the polymerase chain reaction to diagnose *Mycoplasma pneumoniae* infection. Pathology **27:**177–181.
3. **Boom, R., C. J. A. Sol, M. M. M. Salimans, C. L. Jansen, P. M. E. Wertheim-van Dillen, and J. van der Noordaa.** 1990. Rapid and simple method for purification of nucleic acids. J. Clin. Microbiol. **28:**495–503.
4. **Capaul, S. E., and M. Gorgievski-Hrisoho.** 2005. Detection of enterovirus RNA in cerebrospinal fluid (CSF) using NucliSens EasyQ enterovirus assay. J. Clin. Virol. **32:**236–240.
5. **Exner, M. M., and M. A. Lewinski.** 2003. Isolation and detection of Borrelia burgdorferi DNA from cerebal spinal fluid, synovial fluid, blood, urine, and ticks using the Roche magNA Pure system and real-time PCR. Diagn. Microbiol. Infect. Dis. **46:**235–240.
6. **Hoymans, V. Y., J. M. Bosmans, D. Ursi, W. Martinet, F. L. Wuyts, E. Van Marck, M. Altwegg, C. J. Vrints, and M. M. Ieven.** 2004. Immunohistostaining assays for detection of *Chlamydia pneumoniae* in atherosclerotic arteries indicate cross-reactions with nonchlamydial plaque constituents. J. Clin. Microbiol. **42:**3219–3224.
7. **Ieven, M., D. Ursi, H. Van Bever, W. Quint, M. Niesters, and H. Goossens.** 1996. Detection of *Mycoplasma pneumoniae* by two polymerase chain reactions and role of M. pneumoniae in acute respiratory tract infections in pediatric patients. J. Infect. Dis. **173:**1445–1452.
8. **Knepp, J. H., M. A. Geahr, M. S. Forman, and A. Valsamakis.** 2003. Comparison of automated and manual nucleic acid extraction methods for detection of enterovirus RNA. J. Clin. Microbiol. **41:**3532–3536.
9. **Konomi, N., E. Lebwohl, and D. Zhang.** 2002. Comparison of DNA and RNA extraction methods for mummified tissues. Mol. Cell. Probes **16:**445–451.
10. **Lee, B. G., K. R. Fiebelkorn, A. M. Caliendo, and F. S. Nolte.** 2003. Development and verification of an automated sample processing protocol for quantitation of human immunodeficiency virus type 1 RNA in plasma. J. Clin. Microbiol. **41:**2062–2067.
11. **Loens, K., D. Ursi, M. Ieven, P. van Aarle, P. Sillekens, P. Oudshoorn, and H. Goossens.** 2002. Detection of *Mycoplasma pneumoniae* in spiked clinical samples by nucleic acid sequence based amplification. J. Clin. Microbiol. **40:**1339–1345.
12. **Nelissen, R., P. Sillekens, R. Beijer, H. Van Kessel, and W. van Venrooij.** 1991. Structure, chromosomal localization and evolutionary conservation of the gene encoding human U1 snRNP-specific A protein. Gene **102:**189–196.
13. **Rabenau, H. F., A. M. K. Clarici, G. Mûhlbauer, A. Berger, A. Vince, S. Muller, E. Daghofer, B. I. Santner, E. Marth, and H. H. Kessler.** 2002. Rapid detection of enterovirus infection by automated RNA extraction and real-time fluorescence PCR. J. Clin. Virol. **25:**155–164.
14. **Rutjes, S. A., R. Italiaander, H. H. J. L. van den Berg, W. J. Lodder, and A. M. de Roda Husman.** 2005. Isolation and detection of enterovirus RNA from large-volume water samples by using the NucliSens miniMAG system and real-time nucleic acid sequence-based amplification. Appl. Environ. Microbiol. **71:**3734–3740.
15. **Schuurman, T., A. van Breda, R. de Boer, M. Kooistra-Smid, M. Beld, P. Savelkoul, and R. Boom.** 2005. Reduced PCR sensitivity due to impaired DNA recovery with the MagNa Pure LC total nucleic acid isolation kit. J. Clin. Microbiol. **43:**4616–4622.
16. **Smith, K., M. A. Diggle, and S. C. Clarke.** 2003. Comparison of commercial DNA extraction kits for extraction of bacterial genomic DNA from whole-blood samples. J. Clin. Microbiol. **41:**2440–2443.
17. **Stevens, W., T. Wiggil, P. Horsfield, L. Coetzee, and L. E. Scott.** 2005. Evaluation of the NucliSens EasyQ assay in HIV-1 infected individuals in South Africa. J. Virol. Methods **124:**105–110.
18. **Stöcher, M., V. Leb, M. Bozic, H. H. Kessler, G. Halwachs-Baumann, O. Landt, H. Stekel, and J. Berg.** 2003. Parallel detection of five human herpes virus DNAs by a set of real-time polymerase chain reactions in a single run. J. Clin. Virol. **26:**85–93.
19. **Tang, Y.-W., S. E. Sefers, H. Li, D. J. Kohn, and G. W. Procop.** 2005. Comparative evaluation of three commercial systems for nucleic acid extraction from urine specimens. J. Clin. Microbiol. **43:**4830–4833.

20. **Tarp, B., J. S. Jensen, L. Ostergaard, and P. L. Andersen.** 1999. Search for agents causing atypical pneumonia in HIV-positive patients by inhibitor controlled PCR assays. Eur. Respir. J. **13:**175–179.
21. **Tong, C. Y. W., C. Donnelly, G. Harvey, and M. Sillis.** 1999. Multiplex polymerase chain reaction for the simultaneous detection of *Mycoplasma pneumoniae*, Chlamydia pneumoniae and *Chlamydia psittaci* in respiratory samples. J. Clin. Pathol. **52:**257–263.
22. **Ursi, D., K. Dirven, K. Loens, M. Ieven, and H. Goossens.** 2003. Detection of *Mycoplasma pneumoniae* in respiratory samples by real-time PCR using an inhibition control. J. Microbiol. Methods **55:**149–153.
23. **Waring, A. L., T. A. Halse, C. K. Csiza, C. J. Carlyn, K. Arruda Musser, and R. J. Limberger.** 2001. Development of a genomics-based PCR assay for detection of *Mycoplasma pneumoniae* in a large outbreak in New York State. J. Clin. Microbiol. **39:**1385–1390.
24. **Wilson, D., B. Yen-Lieberman, U. Reischl, I. Warshawsky, and G. W. Procop.** 2004. Copmarison of five methods for extraction of *Legionella pneumophila* from respiratory specimens. J. Clin. Microbiol. **42:**5913–5916.
25. **Wolk, D. M., S. K. Schneider, N. L. Wengenack, L. M. Sloan, and J. E. Rosenblatt.** 2002. Real-time PCR method for detection of *Encephalitozoon intestinalis* from stool specimens. J. Clin. Microbiol. **40:**3922–3928.