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Prospective Evaluation of a New Automated Nucleic Acid Extraction System Using Routine Clinical Respiratory Specimens

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The aim of the study was to evaluate the MagNA Pure 96™ nucleic acid extraction system using clinical respiratory specimens for identifying viruses by qualitative real-time PCR assays. Three extraction methods were tested, that is, the MagNA Pure LC™, the COBAS Ampliprep™, and the MagNA Pure 96™ with 10-fold dilutions of an influenza A(H1N1)pdm09 sample. Two hundred thirty-nine respiratory specimens, 35 throat swabs, 164 nasopharyngeal specimens, and 40 broncho-alveolar fluids, were extracted with the MagNA Pure 96™ and the COBAS Ampliprep™ instruments. Forty COBAS Ampliprep™ positive samples were also tested. Real-time PCRs were used to identify influenza A and influenza A(H1N1)pdm09, rhinovirus, enterovirus, adenovirus, varicella zoster virus, cytomegalovirus, and herpes simplex virus. Similar results were obtained on RNA extracted from dilutions of influenza A(H1N1)pdm09 with the three systems: the MagNA Pure LC™, the COBAS Ampliprep™, and the MagNA Pure 96™. Data from clinical respiratory specimens extracted with the MagNA Pure 96™ and COBAS Ampliprep™ instruments were in 98.5% in agreement ($P < 0.0001$) for influenza A and influenza A(H1N1)pdm09. Data for rhinovirus were in 97.3% agreement ($P < 0.0001$) and in 96.8% agreement for enterovirus. They were in 100% agreement for adenovirus. Data for cytomegalovirus and HSV1-2 were in 95.2% agreement ($P < 0.0001$). The MagNA Pure 96™ instrument is easy-to-use, reliable, and has a high throughput for extracting total nucleic acid from respiratory specimens. These extracts are suitable for molecular diagnosis with any type of real-time PCR assay. ***J. Med. Virol. 84: 906–911, 2012.*** © 2012 Wiley Periodicals, Inc.

KEY WORDS: viral diagnosis; nasopharyngeal samples; bronchoalveolar fluids; throat; high throughput

BACKGROUND

The recent emergence of a new strain of influenza, influenza A(H1N1)pdm09, has emphasized the need for a rapid, high throughput system for identifying viruses in respiratory specimens. The diagnosis must also be able to differentiate between influenza A(H1N1)pdm09 itself and other respiratory viruses that may appear as complications. An influenza A(H1N1)pdm09 infection might lead to hospitalization in an intensive care unit where the number of available beds is limited [Fuhrman et al., 2011]. Molecular diagnosis of viral infections has become the "gold standard" in hospital laboratories and has replaced increasingly cellular virus culture and direct immunoassays. While real-time PCRs have improved biological management, the nucleic acid extraction step remains time-consuming and fully automated extraction instruments are needed. High quality real-time PCR assays to identify a broad range of viruses can only be performed on a high quality total nucleic acid extract.

The first automated systems able to extract nucleic acids from various clinical specimens had a limited capacity, no more than 24 or 32 samples. Several high throughput automated platforms are now available. They include the COBAS Ampliprep™ (Roche Diagnostics, Meylan, France) [Sandres-Saune et al., 2007; Schumacher et al., 2007; Sizmann et al., 2007; Alp and Hascelik, 2009], the NucliSENS easyMAG

Abbreviations: IQC, intra-laboratory quality control; FRET, Fluorescence Resonance Energy Transfert; PCR Cp, PCR crossing point.

The authors of this manuscript have no conflicts of interest to disclose.

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(bioMérieux, Boxtel, The Netherlands) [Loens et al., 2007; Perandin et al., 2009; Pillet et al., 2009], the QIAGEN QIAsymphony SP (Qiagen, Valencia, CA) [Miller et al., 2010], and the *m*2000 system (Abbott Laboratories, North Chicago, IL) [Scott et al., 2009]. All of these systems are capable of extracting high quality virus nucleic acid, but they are still time-consuming and require 2–4 hr to extract 96 samples. The recently available MagNA Pure 96™ (Roche Diagnostics) claims to be able to extract nucleic acids from 96 samples in less than 1 hr.

OBJECTIVES

The aim of this study was to examine the performance of the MagNA Pure 96™ system and its convenience for routine use in comparison to the COBAS Ampliprep™.

The first test samples were a series of 10-fold dilutions of influenza A(H1N1)pdm09. They were all extracted using the MagNA Pure LC™, the COBAS Ampliprep™, and the MagNA Pure 96™ instruments (Roche). A series of clinical respiratory specimens were then analyzed using the MagNA Pure 96™ and the COBAS Ampliprep™ instruments by real-time PCRs.

MATERIALS AND METHODS

Materials

Dilutions of a control specimen. The influenza A(H1N1)pdm09 intralaboratory quality control (IQC) was diluted in minimum essential medium to obtain five 10-fold dilutions. This IQC was prepared from infected culture of Madin and Darby canine kidney cells with a clinical respiratory specimen.

The nucleic acids were extracted from each dilution in duplicate using three extraction systems: the MagNA Pure LC™, the COBAS Ampliprep™, and the MagNA Pure 96™.

Routine clinical specimens. A total of 239 clinical respiratory samples were collected prospectively during 1 week between January 27, 2010 and February 4, 2010 by the Department of Virology, CHU Toulouse, France. The nucleic acids were extracted from these samples using the COBAS Ampliprep™ and the MagNA Pure 96™ instruments.

They were 35 throat samples collected on virologically adapted Virocult® (Kitvia, Labarthe Inard, France), 164 nasopharyngeal swab samples collected on Virocult®, and 40 broncho-alveolar fluids collected in sterile tubes.

Samples were tested for several viruses, including influenza A, influenza A(H1N1)pdm09, rhinovirus, enterovirus, adenovirus, varicella zoster virus, cytomegalovirus, and herpes simplex type 1 (Table I).

COBAS Ampliprep™ selected positive samples. Respiratory clinical samples tested positive for adenovirus (N = 15), cytomegalovirus (N = 9), enterovirus (N = 9), and HSV (N = 7) were also tested. They were nasopharyngeal specimens (adenovirus

TABLE I. Real-Time Molecular Tests Used for the Identification of Viruses Extracted From Clinical Respiratory Specimens

Virus	Specimens (n)	Type of specimens	PCR chemistry	Molecular test	Real-time instrument	Refs.
Influenza A and A(H1N1)pdm09	64	Throat sample: 1; nasopharyngeal: 60; bronchoalveolar fluid: 3	Hydrolysis probe	Commercial kit: RealTime ready Inf A/H1N1 Detection Set®	Light Cycler 480	
Rhinovirus	74	Nasopharyngeal: 67; bronchoalveolar fluid: 7	SYBR Green + melting curve	Home-brew	Light Cycler 2.0	Kares et al. [2004]
Enterovirus	31	Throat sample: 10; nasopharyngeal: 21	Hydrolysis probe	Home-brew	Light Cycler 2.0	Verstrepen et al. [2002]
Adenovirus	41	Nasopharyngeal: 39; bronchoalveolar fluid: 2	Hydrolysis probe	Home-brew	Light Cycler 2.0	Heim et al. [2003]
Varicella zoster virus	6	Bronchoalveolar fluid: 6	FRET	Home-brew	Light Cycler 2.0	Espy et al. [2000]
Cytomegalovirus	21	Nasopharyngeal: 1; bronchoalveolar fluid: 20	Hydrolysis probe	Home-brew	Light Cycler 2.0	Mengelle et al. [2003]
Herpes simplex virus 1 and 2	42	Throat sample: 24; bronchoalveolar fluid: 18	FRET + melting curve	Home-brew	Light Cycler 1.0	Mengelle et al. [2004]

FRET, Fluorescence Resonance Energy Transfert.

and enterovirus) and bronchoalveolar fluids (cytomegalovirus and HSV).

Extraction

Three systems were used: the MagNA Pure LC Total Nucleic Kit® (Roche) on the MagNA Pure LC™ instrument, the COBAS Ampliprep Total Nucleic Acid Isolation kit® (TNAI; Roche) on the COBAS Ampliprep™ instrument [Mengelle et al., 2008], and the MagNA Pure 96 DNA and Viral NA Small Volume Kit® (Roche) on the MagNA Pure 96™.

Input and output volumes were 200 and 100 μl on the MagNA Pure™ systems. The volumes were 500 and 75 μl on the COBAS Ampliprep™.

Molecular Amplification Tests

PCR tests used are described in Table I. Influenza A and influenza A(H1N1)pdm09 viruses were detected with the RealTime ready Inf A/H1N1 Detection Set® with the RNA virus master® according to manufacturer's instructions on the Light Cycler 480™ system (Roche Diagnostics). The set contained specific primer/probe mixes for detecting influenza A matrix protein 2 (M2) and the Mexico variant specific hemagglutinin HA1 (H1) in a single pass.

Rhinovirus was detected on the Light Cycler 2.0™ real-time instrument with an in-house technique [Kares et al., 2004]. Samples underwent one step RT-PCR using the SYBR Green technique, and then melting curve analysis was used to identify rhinovirus.

Enterovirus [Verstrepen et al., 2002], adenovirus [Heim et al., 2003], varicella zoster virus [Espy et al., 2000], and cytomegalovirus [Mengelle et al., 2003] were detected on the Light Cycler 2.0™ using in-house real-time PCRs.

Herpes simplex types 1 and 2 were detected on the Light Cycler 1.0 ™, as previously described [Mengelle et al., 2004]. FRET technology was used on the melting curve analysis to differentiate between HSV-1 and HSV-2.

Statistical Analysis

Data were analyzed using Stata™ software 9.2 (StataCorp, College Station, TX). The Kappa Cohen test was used to compare the performances of the assays. A P-value of <0.05 was considered significant.

RESULTS

Analytical Performances Using a Control Specimen

The five 10-fold dilutions of the IQC influenza A(H1N1)pdm09 were extracted each twice with the three extraction instruments, the MagNA Pure LC™, the COBAS Ampliprep™, and the MagNA Pure 96™. Each extract was tested for influenza A (target M2) and influenza A(H1N1)pdm09 (target H1).

Influenza A and influenza A(H1N1)pdm09 viruses were detected in RNA extracted with all the three instruments from the undiluted IQC and in the 10^{-1}, 10^{-2}, and 10^{-3} dilutions.

Both targets were detected in both 10^{-4} dilutions extracted with the MagNA Pure LC™ and the MagNA Pure 96™ (Table II), but only every other target was detected in 10^{-4} dilutions extracted with the COBAS Ampliprep™ instrument.

The H1 target was detected once, in one 10^{-5} dilution extracted with the MagNA Pure LC™ instrument. Both targets were negative in 10^{-5} dilutions extracted with the MagNA Pure 96™ and the COBAS Ampliprep™ instruments.

TABLE II. Detection of Influenza A (Target M2) and Influenza AH1N1(2009) (Target H1) on Extracts of 10-Fold Dilutions of an Influenza AH1N1(2009) Virus Culture With the MagNA Pure LC™, the COBAS Ampliprep™, and the MagNA Pure 96™ Systems

	MagNA Pure LC™		COBAS Ampliprep™		MagNA Pure 96™	
	M2 target	H1 target	M2 target	H1 target	M2 target	H1 target
Influenza AH1N1(2009) Detection Set®	PCR Cp	PCR Cp	PCR Cp	PCR Cp	PCR Cp	PCR Cp
Pure	21.98	21.4	27.47	24.13	24.29	22.35
Pure	21.92	21.11	26.63	24	24.5	22.55
10^{-1}	25.24	24.4	30.48	27.85	27.26	25.29
10^{-1}	25.23	24.36	30.37	27.91	27.22	25.49
10^{-2}	28.65	27.95	34.49	30.88	31.53	29.62
10^{-2}	28.34	26.80	33.55	30.49	31.89	29.37
I10^{-3}	32.43	31.37	36.89	33.71	35.58	33.33
10^{-3}	31.55	30.99	35.63	34.63	35.51	33.86
10^{-4}	36.27	34.52	35.40	Negative	39.07	36.34
10^{-4}	33.71	34.73	Negative	39.64	40.34	37.49
10^{-5}	Negative	39.53	Negative	Negative	Negative	Negative
10^{-5}	Negative	Negative	Negative	Negative	Negative	Negative

PCR Cp, PCR crossing point.

TABLE III. Detection of RNA Viruses

	COBAS Ampliprep™											
	RealTime ready Inf A/H1N1 Detection Set®			RealTime ready Inf A/H1N1 Detection Set®			Rhinovirus			Enterovirus		
	Target M			Target H1								
	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total
MagNA Pure 96™												
Positive	3	1[a]	4	2	1[b]	3	16	2	18	10	0	10
Negative	0	60	60	0	61	61	0	56	56	1	20	21
Total	3	61	64	2	62	64			74	11	20	31

[a]Sample negative for H1.
[b]Sample positive for M2 with both extraction systems.

Analytical Performances Using Routine Clinical Samples

Two hundred thirty-nine clinical respiratory samples were extracted prospectively with the COBAS Ampliprep™ and the MagNA Pure 96™ instruments. As the number of positive samples was too small, 40 additional COBAS Ampliprep™ positive samples were extracted with the MagNA Pure 96™ instrument. Prospective samples and retrospective positive samples were pooled for analysis.

Detection of RNA viruses. Sixty-four samples were tested for influenza A and influenza A(H1N1) pdm09. Three samples were influenza A (M2) positive, whereas 60 were negative. One nasopharyngeal sample was M2 positive with the MagNA Pure 96™ and M2 negative with the COBAS Ampliprep™.

Two samples were influenza A(H1N1)pdm09 positive, and 61 were negative by both extraction methods. One nasopharyngeal sample was positive for H1 after MagNA Pure 96™ extraction and negative after COBAS Ampliprep™ extraction. The detections of influenza A and influenza A(H1N1)pdm09 were in 98.5% concordance ($P < 0.0001$).

Seventy-four samples were tested for rhinovirus: 16 samples were positive and 56 negative with both extraction systems. Two samples were MagNA Pure 96™ positive and COBAS Ampliprep™ negative. The detections were in 97.3% concordance ($P < 0.0001$).

The results for enterovirus agreed 96.8%: 10 samples were positive and 20 were negative. Both techniques disagreed on one sample which was MagNA Pure 96™ negative (Table III).

Detection of DNA viruses. The two extraction systems agreed 100% for adenovirus: 15 samples were positive and 26 samples were negative, and for varicella zoster virus: six negative samples.

Twenty-one broncho-alveolar samples were tested for cytomegalovirus: 10 were negative and 10 were positive by both methods. One sample was MagNA Pure 96™ positive and COBAS Ampliprep™ negative. There was thus 95.2% concordance ($P < 0.0001$).

Detection of HSV1-2 gave 95.2% concordant results: 40 samples gave identical results, 10 samples were positive, and 30 were negative ($P < 0.0001$). The 10 positive results gave identical herpes virus types (HSV-1). One sample was MagNA Pure 96™ positive/ COBAS Ampliprep™ negative and one sample gave the opposite result. Both samples were broncho-alveolar specimens containing very little herpes simplex type 1 (Table IV).

Practicality. The MagNA pure 96™ and the COBAS Ampliprep™ use the same technology: lysis to release nucleic acids that are then bound to the silica surface of added magnetic particles due to the chaotropic salt conditions and the high ionic strength of the lysing reagent.

The reagents supplied with both instruments are ready-to-use and may be kept at room temperature for about 30 days.

However, the instruments are used very differently: samples are dispensed in steps of 24 samples onto the

TABLE IV. Detection of DNA Viruses

	COBAS Ampliprep™											
	Adenovirus			Zoster virus			Cytomegalovirus			Herpes simplex virus		
	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total
MagNA Pure 96™												
Positive	15	0	0	0	0	0	10	1	11	10	1	11
Negative	0	26	26	0	6	6	0	10	10	1	30	31
Total	15	26	41	0	6	6	10	11	21	11	31	42

COBAS Ampliprep™, so that extraction of 96 samples takes at least 4 hr. The MagNA Pure 96™ instrument uses a 96-nozzle pipette head and 8–96 samples can be processed in a single run of less than 1 hr. The MagNA pure 96™ also requires an input volume of 200 μl, and elution provides 100 μl. Most real-time PCR tests require an input volume of 5–10 μl, so at least seven viruses can be detected in a single extract.

DISCUSSION

The performance of a new commercially available high throughput extraction system was evaluated and was compared with other, older systems on a standard sample and on routine clinical samples.

Similar results were obtained when RNA was extracted from dilutions of influenza A(H1N1)pdm09 with three systems, the MagNA Pure LC™, the COBAS Ampliprep™, and the MagNA Pure 96™. Previous studies on the quantification of EBV [Mengelle et al., 2008] and CMV [Mengelle et al., 2011] in whole blood found a good correlation between the MagNA Pure LC™ system and the COBAS Ampliprep™. Thus, these data show the importance of comparing the new extraction system, the MagNA Pure 96™, with another highly automatic instrument that uses identical technology, the COBAS Ampliprep™. For this purpose, total virus nucleic acid (DNA and RNA) was extracted from respiratory specimens with both instruments and then tested for several viruses (influenza A and influenza A(H1N1)pdm09, rhinovirus, enterovirus, adenovirus, varicella zoster virus, cytomegalovirus, and herpes virus types 1 and 2) by real-time PCRs.

Previous studies have shown that high throughput automated extraction systems can be used for nasopharyngeal aspirates [Chan et al., 2008], plasma [Miller et al., 2010], and EDTA whole blood samples [Pillet et al., 2009]. Chan et al. compared two automatic extraction systems (the Nuclisens easyMAG and the Qiagen BioRobot 9604) with the manual QIAamp (Qiagen) extraction method. They found no difference in the sensitivities of all three methods. Furthermore, until recently the MagNA Pure LC™ system has provided an easy-to-use platform for extracting various clinical specimens. But we believe this is the first report of using the MagnA Pure 96™ instrument to extract nucleic acids from a large number of routine clinical respiratory specimens, including broncho-alveolar fluids, throat samples, and nasopharyngeal samples that were collected in 1 week during the influenza A(H1N1)pdm09 pandemic. The results show that the two extraction systems performed very similarly on all the clinical respiratory specimens regardless the real-time PCR technology used. Moreover, the methods performed similarly regardless of the structure of the viruses (DNA and RNA, with or without an envelope). The viruses tested can serve as models for all other viruses, including influenza B, respiratory syncytial virus, and coronaviruses. Some of theses viruses, such as varicella zoster virus, are rarely found in respiratory infections, but as this was a prospective study, all the items requested by the clinicians were tested.

The use of this new automated system entails no change in sample preparation. Samples are diluted and mixed in minimum essential medium before extraction, and no extra medium, such as N-acetyl cysteine, is necessary.

The MagNA Pure 96™ can extract 96 samples in less than 1 hr and several extractions can be run in a single day if necessary. The advent of the MagNA Pure 96™ has lead to huge changes in the organization of the laboratory. Two extractions a day are now run, so clearing all daily incoming clinical specimens. In addition, as elution volume is 100 μl, only a single extraction is needed even if several PCR tests are to be done. This greatly reduces the technician's hands-on time and hence the cost of the entire PCR test. As most PCR assays are now carried out on the same day, the waiting time for results has decreased dramatically, as have the number of phone calls from clinicians.

The MagNA Pure 96™ software is easy-to use, so that technicians do not require a long initial training. Maintenance is also easy; it requires only a few minutes a day.

In conclusion, the MagNA Pure 96™ high throughput automated extraction system is a reliable device for extracting total virus nucleic acids from a broad range of clinical respiratory specimens in order to detect viruses by various real-time PCR tests. A batch of 96 samples can be extracted in less than 1 hr, which allows for very rapid molecular diagnosis and thus identification of viral pathogens. Operator time is reduced thanks to ready-to use reagents that can be kept at room temperature. These features are all of great economic importance, in addition to providing rapid diagnosis and improving patient management.

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REFERENCES

Alp A, Hascelik G. 2009. Comparison of 3 nucleic acid isolation methods for the quantification of HIV-1 RNA by Cobas Taqman real-time polymerase chain reaction system. Diagn Microbiol Infect Dis 63:365–371.

Chan KH, Yam WC, Pang CM, Chan KM, Lam SY, Lo KF, Poon LL, Peiris JS. 2008. Comparison of the NucliSens easyMAG and Qiagen BioRobot 9604 nucleic acid extraction systems for detection of RNA and DNA respiratory viruses in nasopharyngeal aspirate samples. J Clin Microbiol 46:2195–2199.

Espy MJ, Teo R, Ross TK, Svien KA, Wold AD, Uhl JR, Smith TF. 2000. Diagnosis of varicella-zoster virus infections in the clinical laboratory by LightCycler PCR. J Clin Microbiol 38:3187–3189.

Fuhrman C, Bonmarin I, Bitar D, Cardoso T, Duport N, Herida M, Isnard H, Guidet B, Mimoz O, Richard JC, Brun-Buisson C, Brochard L, Mailles A, Paty AC, Saura C, Levy-Bruhl D. 2011.

Adult intensive-care patients with 2009 pandemic influenza A(H1N1) infection. Epidemiol Infect 139:1202–1209.

Heim A, Ebnet C, Harste G, Pring-Akerblom P. 2003. Rapid and quantitative detection of human adenovirus DNA by real-time PCR. J Med Virol 70:228–239.

Kares S, Lonnrot M, Vuorinen P, Oikarinen S, Taurianen S, Hyoty H. 2004. Real-time PCR for rapid diagnosis of entero- and rhinovirus infections using LightCycler. J Clin Virol 29:99–104.

Loens K, Bergs K, Ursi D, Goossens H, Ieven M. 2007. Evaluation of NucliSens easyMAG for automated nucleic acid extraction from various clinical specimens. J Clin Microbiol 45:421–425.

Mengelle C, Sandres-Saune K, Pasquier C, Rostaing L, Mansuy JM, Marty M, Da Silva I, Attal M, Massip P, Izopet J. 2003. Automated extraction and quantification of human cytomegalovirus DNA in whole blood by real-time PCR assay. J Clin Microbiol 41:3840–3845.

Mengelle C, Sandres-Saune K, Miedouge M, Mansuy JM, Bouquies C, Izopet J. 2004. Use of two real-time polymerase chain reactions (PCRs) to detect herpes simplex type 1 and 2—DNA after automated extraction of nucleic acid. J Med Virol 74:459–462.

Mengelle C, Legrand-Abravanel F, Mansuy JM, Barthe C, Da Silva I, Izopet J. 2008. Comparison of two highly automated DNA extraction systems for quantifying Epstein–Barr virus in whole blood. J Clin Virol 43:272–276.

Mengelle C, Mansuy JM, Da Silva I, Davrinche C, Izopet J. 2011. Comparison of 2 highly automated nucleic acid extraction systems for quantitation of human cytomegalovirus in whole blood. Diagn Microbiol Infect Dis 69:161–166.

Miller S, Seet H, Khan Y, Wright C, Nadarajah R. 2010. Comparison of QIAGEN automated nucleic acid extraction methods for CMV quantitative PCR testing. Am J Clin Pathol 133:558–563.

Perandin F, Pollara PC, Gargiulo F, Bonfanti C, Manca N. 2009. Performance evaluation of the automated NucliSens easyMAG nucleic acid extraction platform in comparison with QIAamp Mini kit from clinical specimens. Diagn Microbiol Infect Dis 64: 158–165.

Pillet S, Bourlet T, Pozzetto B. 2009. Comparative evaluation of a commercially available automated system for extraction of viral DNA from whole blood: application to monitoring of Epstein–Barr virus and cytomegalovirus load. J Clin Microbiol 47:3753–3755.

Sandres-Saune K, Abravanel F, Nicot F, Peron JM, Alric L, Boineau J, Pasquier C, Izopet J. 2007. Detection and quantitation of HCV RNA using real-time PCR after automated sample processing. J Med Virol 79:1821–1826.

Schumacher W, Frick E, Kauselmann M, Maier-Hoyle V, van der Vliet R, Babiel R. 2007. Fully automated quantification of human immunodeficiency virus (HIV) type 1 RNA in human plasma by the COBAS AmpliPrep/COBAS TaqMan system. J Clin Virol 38: 304–312.

Scott LE, Noble LD, Moloi J, Erasmus L, Venter WD, Stevens W. 2009. Evaluation of the Abbott m2000 RealTime human immunodeficiency virus type 1 (HIV-1) assay for HIV load monitoring in South Africa compared to the Roche Cobas AmpliPrep-Cobas Amplicor, Roche Cobas AmpliPrep-Cobas TaqMan HIV-1, and BioMerieux NucliSENS EasyQ HIV-1 assays. J Clin Microbiol 47:2209–2217.

Sizmann D, Boeck C, Boelter J, Fischer D, Miethke M, Nicolaus S, Zadak M, Babiel R. 2007. Fully automated quantification of hepatitis C virus (HCV) RNA in human plasma and human serum by the COBAS AmpliPrep/COBAS TaqMan system. J Clin Virol 38:326–333.

Verstrepen WA, Bruynseels P, Mertens AH. 2002. Evaluation of a rapid real-time RT-PCR assay for detection of enterovirus RNA in cerebrospinal fluid specimens. J Clin Virol 25:S39–S43.