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[▽]

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The NucliSens easyMAG and BioRobot 9604 automated nucleic acid extraction systems were evaluated and compared with the manual QIAamp (Qiagen) extraction method for their abilities to extract nucleic acid from nasopharyngeal aspirate samples for the detection of RNA and DNA respiratory viruses. The nucleic acids recovered by all three methods gave comparable sensitivities in PCR tests, and the three methods gave comparable viral loads. There was no evidence of residual PCR inhibitors and no evidence of PCR cross-contamination.

Nucleic acid amplification techniques are becoming widely used for virus detection in clinical virology, as they combine high sensitivity, high specificity, the possibility of their use for quantitation or multiplexing, and the rapid turnaround of results. They are able to detect a range of viral infections affecting the respiratory tract and the central nervous system, blood-borne viruses, sexually transmitted pathogens, and viruses causing disease in immunocompromised hosts, such as cytomegalovirus (18). The emergence of viral respiratory disease outbreaks such as severe acute respiratory syndrome and the impending pandemic threat posed by avian influenza virus H5N1 highlight the need to have available reliable medium- or high-throughput platforms for the detection of respiratory viral disease threats (13, 12, 7).

A reliable automated method for the extraction of high-quality nucleic acid from clinical specimens is an essential part of providing this medium- to high-throughput detection capacity. Boom and colleagues (3) developed an extraction method which can purify nucleic acid and efficiently remove PCR inhibitors from various clinical samples so that the nucleic acid has a high sensitivity and a high specificity in PCR assays. Several robotic platforms based on this principle are now available for the purification of nucleic acid from patient samples for molecular detection and include the BioRobot EZ1 system (low throughput; Qiagen GmbH, Hilden, Germany), the MagNA Pure LC instrument (medium throughput; Roche Diagnostics GmbH, Germany), and the BioRobot 9604 system (high throughput; Qiagen GmbH). A study showed that the miniMAG manual extraction system (bioMérieux, The Netherlands) produced the largest quantity of total nucleic acids and the best precision compared to the quantities and preci-

sions produced by the MagNA Pure compact system (Roche Diagnostics GmbH) and the BioRobot EZ1 system when nucleic acids were extracted from urine specimens (16). Recently, the medium-throughput automated NucliSens easyMAG nucleic acid extraction system (bioMérieux) has become available. This system has been evaluated for its ability to monitor human immunodeficiency virus loads (14). A comparison of the NucliSens easyMAG system with the Nuclisens miniMAG and Qiagen QIAamp blood kit with throat swab specimens for the detection of *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* has been reported, although the study was based on a limited number ($n = 15$) of positive swab specimens (8). In the study described here, we compared the performance, quality, and quantity of total nucleic acid extracted from respiratory specimens with two automated systems, the NucliSens easyMAG system (bioMérieux) and the BioRobot 9604 kit (Qiagen GmbH), with those of total nucleic acid extracted from respiratory specimens with the manual QIAamp RNA and DNA extraction kits (Qiagen GmbH) for the detection of RNA and DNA viral respiratory pathogens.

MATERIALS AND METHODS

Respiratory samples. The evaluation was carried out from February to April 2006 with 200 nasopharyngeal aspirate (NPA) samples submitted to the Department of Microbiology, Queen Mary Hospital, Hong Kong, for the clinical detection of respiratory viral pathogens. Two aliquots were prepared. One was used for viral culture (6) and another was used for respiratory virus detection by a direct immunofluorescent (DIF) test for respiratory viruses (influenza A virus, influenza B virus, respiratory syncytial virus [RSV], adenovirus, parainfluenza virus type 1, parainfluenza virus type 2, and parainfluenza virus type 3) (5, 6) and by PCR methods. In addition, 86 stored NPA samples known to be positive for influenza A virus ($n = 30$), RSV ($n = 30$), and adenovirus ($n = 25$) collected in 2005 were used for viral load comparisons.

Automated nucleic acid extraction methods. Total nucleic acid extraction was performed by using the NucliSens easyMAG instrument (bioMérieux), according to the manufacturer's instructions. Briefly, 250 μ l of an NPA sample was added to 2 ml of lysis buffer and the mixture was incubated for 10 min at room temperature. The lysed sample was then transferred to the well of a plastic vessel with 100 μ l of silica. This was followed by automatic magnetic separation. Nucleic acid was recovered in 55 μ l elution buffer.

By using a parallel specimen, nucleic acid was also extracted with the Bio-

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FIG. 1. Comparison of viral loads of influenza A virus (a), RSV (b), and adenovirus (c) in nucleic acid extracted from NPA specimens by the automated easyMAG system and BioRobot 9604 kit and the manual Qiagen RNA kit.

Robot 9604 kit (Qiagen GmbH), according to the manufacturer's instructions. Briefly, 220 μ l of an NPA sample was added to 280 μ l of lysis buffer containing carrier RNA and the mixture was incubated at 60°C in a dry bath for 10 min. The mixture with 275 μ l ethanol was passed through a 96-well QIAamp plate by vacuum suction and washed three times in washing buffer. The final elution volume was 86 μ l.

RNA and DNA extraction by manual QIAamp method. For RNA and DNA extraction, the QIAamp viral RNA kit (Qiagen GmbH) with 140 μ l of an NPA sample and the QIAamp DNA kit (Qiagen GmbH) with 200 μ l of an NPA sample, respectively, were used according to the manufacturer's instructions. The final elution volumes of RNA and DNA were 60 μ l and 200 μ l, respectively.

Qualitative PCR. Influenza A virus, influenza B virus, and RSV were amplified by a multiplex PCR as described previously (15). Another set of multiplex primers was used to amplify the hemagglutinin-neuraminidase gene of parainfluenza virus types 1, 2, and 3 or the phosphoprotein gene of parainfluenza virus

type 4 (1). Adenovirus was amplified by nested PCR with primers targeting the hexon gene (10).

Quantitative PCRs. Quantitative PCRs for RNA viruses (influenza A virus and RSV) and a DNA virus (adenovirus) were performed by previously described methods (6). Briefly, 2 μ l eluted RNA of influenza A virus or RSV was used for the collection of cDNA by use of the Invitrogen Superscript II kit with random primers, as described previously (11), and then the cDNA was amplified in a LightCycler instrument with a FastStart DNA master SYBR green I mix reagent kit (Roche Diagnostics GmbH). In a typical reaction, 2 μ l cDNA was amplified in 20 μ l of the LightCycler PCR master mixture containing 1 $\times$ FastStart DNA master SYBR green I mix, 4.0 mM MgCl₂, and 0.5 μ M each primer.

For the quantitative PCR for adenovirus, 5 μ l of the nucleic acid template was added to 20 μ l of the master mixture containing 1 $\times$ FastStart DNA master SYBR green I mix (Roche Diagnostics GmbH), 3.0 mM MgCl₂, and 0.25 μ M each primer. To determine the specificity of the assay, all PCR products were

TABLE 1. Comparison of numbers of viral copies recovered by automatic or manual extraction methods from stored nasopharyngeal extract specimens

Virus	No. of samples	Mean ($\pm$ SD) viral load (no. of copies/ml)		
		easyMAG system	BioRobot 9604 kit	Qiagen RNA kit
Influenza A virus	30	$5.2 \times 10^8 (\pm 7.6 \times 10^8)$	$6.3 \times 10^8 (\pm 1.6 \times 10^9)$	$5.7 \times 10^8 (\pm 9.0 \times 10^8)$
RSV	30	$1.4 \times 10^{10} (\pm 6.9 \times 10^{10})$	$1.4 \times 10^9 (\pm 2.7 \times 10^9)$	$1.7 \times 10^9 (\pm 4.0 \times 10^9)$
Adenovirus	25	$1.0 \times 10^8 (\pm 1.8 \times 10^8)$	$4.1 \times 10^7 (\pm 7.2 \times 10^7)$	$9.4 \times 10^7 (\pm 2.3 \times 10^8)$

subjected to melting curve analysis (65°C to 95°C; 0.1°C per s) at the end of the assay.

For each quantitative assay, a reference standard was prepared with the pC-RII-TOPO vector (Invitrogen, San Diego, CA) containing the corresponding target viral sequences. A series of 5 log₁₀ dilutions equivalent to 1×10^1 to 1×10^6 copies per reaction mixture were prepared to generate calibration curves and were run in parallel with the test samples. If the result for the specimen was outside the upper limit of the expected range, a suitable dilution of an extract of the sample was retested.

Quality control of specimens and PCR. To assess the quality of the DNA extracted from clinical specimens, the specimens were evaluated for the presence of human DNA by a PCR assay targeting the human mitochondrial cytochrome oxidase (HMCO) gene, which amplifies a 823-bp product of the HMCO gene, as described previously (4). In order to prevent PCR cross-contamination, separate rooms were used to perform the PCR procedures, including nucleic acid extraction, preparation of PCR reagents, setting up of the PCRs, PCR amplification, and post-PCR analysis. Positive and multiple negative controls were included in each PCR run.

RESULTS

No significant inhibition (threshold cycle range, 31.28 to 32.92) by the nucleic acid extracts prepared with both automated extraction systems was found when plasmids containing 100 copies each of RSV and adenovirus nucleic acids were separately spiked into 60 randomly chosen extracted negative samples. These negative samples contained comparable nucleic acid concentrations (dilution range, 3 to 3.5 log₁₀) of the HMCO gene by PCR.

In a retrospective evaluation of 86 stored NPA specimens confirmed to be virus positive by culture (30 positive for influenza A virus, 30 positive for RSV, and 25 positive for adenovirus), nucleic acid was extracted by each of the three methods and the viral loads were compared. The mean viral loads per ml of sample extracted by the easyMAG system, the BioRobot 9604 kit, and the Qiagen RNA kit for influenza A virus, RSV, and adenovirus were not significantly different (Fig. 1; Table 1).

In a prospective study, 200 freshly collected NPA specimens submitted to Queen Mary Hospital from February to April 2006 were investigated for virus detection. Forty-six of these were found to be positive by the DIF test for respiratory viruses (13 for influenza A virus, 14 for influenza B virus, 16 for RSV, 1 for adenovirus, and 2 for parainfluenza virus type 1) and 47 were culture positive (11 for influenza A virus, 16 for influenza B virus, 14 for RSV, 3 for adenovirus, and 3 for parainfluenza virus type 1). Seventy-four of the nucleic acid extracts obtained with the NucliSens easyMAG system and 76 of the nucleic acid extracts obtained with the BioRobot 9604 kit were found to be positive for respiratory viruses by reverse transcription-PCR and PCR methods. The results obtained by the different tests and the different extraction methods are compared in Table 2.

If any two tests were positive for the same virus, the result

was considered true positive. On the basis of this definition, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of PCR assays done with RNA extracted with the easyMAG system and the BioRobot 9604 kit are shown in Table 3. The only notable differences between the two methods were the sensitivity for influenza A virus, in which RNA extraction with the easyMAG system yielded a sensitivity of 88.2% (15/17 specimens were positive), whereas RNA extraction with the BioRobot9604 kit yielded a sensitivity of 100% (17/17 specimens were positive) with the same clinical specimens and by the same reverse transcription-PCR method. However, these differences were not significantly different (chi-square test, $P = 0.89$).

Two specimens, including one containing influenza A virus and one containing adenovirus, were found to be negative by both culture and DIF but positive by single PCR (Table 2). These single-PCR-positive samples were consistently found to be positive when RNA extraction from the original samples and PCR were repeated. Therefore, the results for these two

TABLE 2. Comparison of the performance characteristics of two extraction methods for PCR, DIF, and culture for detection of respiratory viruses

Virus	Result by:				Total no. of specimens
	Culture	DIF test	PCR		
			easyMAG system	BioRobot 9604 kit	
Influenza A virus	+	+	+	+	8
	+	+	—	+	2
	+	—	+	+	1
	—	+	+	+	3
	—	—	+	+	3
	—	—	+	—	1
Influenza B virus	+	+	+	+	12
	+	—	+	+	4
	—	+	+	+	2
	—	—	+	+	4
RSV	+	+	+	+	12
	+	—	+	+	2
	—	+	+	+	4
	—	—	+	+	2
Parainfluenza virus type 1	+	+	+	+	2
	+	—	+	+	1
	—	—	+	+	2
Adenovirus	+	+	+	+	1
	+	—	+	+	2
	—	—	+	+	8
	—	—	—	+	1

TABLE 3. Performance characteristics of different assays for detection of respiratory viruses by use positive results by any two tests as a true-positive result

Assay	Influenza A virus (<i>n</i> = 18)					Influenza B virus (<i>n</i> = 22)					RSV (<i>n</i> = 20)					Parainfluenzavirus type 1 (<i>n</i> = 5)					Adenovirus (<i>n</i> = 12)				
	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	
PCR (easyMAG system)	88.20	99.50	93.80	98.90	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
PCR (BioRobot 9604 kit)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	91.70	99.50	91.70	100	100
Culture	64.70	100	100	96.80	72.70	100	100	100	96.70	70.00	100	100	100	100	96.80	60.00	100	100	100	99.00	27.00	100	100	100	95.90
DIF test	76.40	100	100	97.90	63.60	100	100	100	95.70	80.00	100	100	100	100	97.80	40.00	100	100	100	98.50	9.00	100	100	100	95.00

positive samples were unlikely to be false positive. If we take the results for these two samples positive by PCR to be true positive, then the sensitivity, specificity, PPV, and NPV of the NucliSens easyMAG system become 88.9%, 100%, 100%, and 98.9%, respectively, for influenza A virus and 91.7%, 100%, 100%, and 99.5%, respectively, for adenovirus, while those of the BioRobot 9604 kit become 94.4%, 100%, 100%, and 99.5%, respectively, for influenza A virus and 100%, 100%, 100%, and 100%, respectively, for adenovirus. Nevertheless, these differences in sensitivity were statistically insignificant (chi-square test, *P* = 0.88 and 0.71, respectively).

DISCUSSION

The performance of the easyMAG automated nucleic acid extraction system was compared with that of the BioRobot 9604 kit and the manual Qiagen RNA extraction kit for the extraction of nucleic acids for use for the PCR detection of two RNA respiratory viruses (influenza A virus and RSV) and a DNA virus (adenovirus). The viral loads in stored NPA specimens were studied retrospectively. Although there were marginal differences in the viral loads in the nucleic acid extracts obtained by these different methods for individual viruses, no statistically significant differences between the viral loads in nucleic acid extracted with the easyMAG system and the BioRobot 9604 kit, the easyMAG system and the Qiagen kit, and the BioRobot 9604 kit and the Qiagen kit were found: for influenza A virus, *P* = 0.74, 0.86, and 0.82, respectively; for RSV, *P* = 0.32, 0.78, and 0.32, respectively; and for adenovirus, *P* = 0.13, 0.29, and 0.89, respectively (Fig. 1; Table 1).

There was no evidence of PCR inhibitors or cross-contamination in the nucleic acid extracts obtained by either automated method. If a specimen with positive results for the same virus by any two tests was used as the “gold standard,” both the automated easyMAG system and the BioRobot 9604 kit had excellent and comparable sensitivities when the nucleic acids obtained were used for the PCR detection of these respiratory viruses. While the sensitivities of detection of influenza A virus with RNA extracted with the easyMAG system and the BioRobot 9604 kit were 88.9% and 94.4%, respectively, this difference was not statistically significant (chi-square test, *P* = 0.86).

Several studies previously showed that multiplex PCRs for respiratory viruses are rapid, accurate, and sensitive for the detection of respiratory viral pathogens (2, 15, 17). In this study, we also see that PCR methods have markedly higher sensitivities than culture and the DIF test for the detection of respiratory viruses (Table 3). The sensitivities of culture for the detection of influenza A or B virus, RSV, or parainfluenza virus type 1 ranged from 60 to 72% and those of the DIF test ranged from 63 to 80%. PCR methods also have the advantage of being able to detect a number of viral respiratory pathogens that are difficult to culture (e.g., human metapneumovirus and coronaviruses). On the other hand, while culture and the DIF test were markedly less sensitive at detecting adenovirus (29% and 9%, respectively), it must be noted that adenovirus may be detected in the upper respiratory tract for many months and its detection is not evidence of clinical significance. Indeed, this may be a situation in which the less sensitive DIF test method provides a better indication of clinical significance (9). In this study, we found that the mean viral load for adenovirus in

culture-positive and DIF test-positive specimens (9.9×10^7) was statistically significantly higher ($P = 0.01$) than that in culture-negative and DIF test-negative specimens (5.9×10^5).

As PCR and multiplex PCR become more widely used for the clinical detection of viruses, high-throughput and automated methods of nucleic acid extraction become increasingly important. We report here that in comparison with manual nucleic acid extraction methods, these two automated methods provide satisfactory results.

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