Automated nucleic acid isolation in viral molecular diagnostics: evaluation of the QIAsymphony SP

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ABSTRACT

The Qiagen QIAsymphony SP is a high-throughput (up to 96 samples per run), fully-automated nucleic acid isolation system. It was implemented in the authors' laboratory to cope with the high demand for pandemic H1N1 influenza testing in 2009. This study evaluated the QIAsymphony SP for viral nucleic acid isolation from quality control materials, pure cultures and various clinical specimens. The effect of varying sample volume on detection sensitivity was investigated using serial 10-fold dilutions of pure viral specimens and target nucleic acids were detected by real-time polymerase chain reaction (PCR) assays. Little variability in detection sensitivity was observed for all the viral targets tested, although variation in cycle threshold values was apparent in some cases. Importantly, pathogens were detectable over a broad concentration range and from diverse clinical specimens. Removal of PCR inhibitors was generally effective, as demonstrated by detection of viral nucleic acids and/or internal controls. The results demonstrate that the QIAsymphony SP is suitable for use in routine virology molecular diagnostics, and provides a high-throughput capacity, which is needed in peak seasons of infection or in centralised laboratories.

KEY WORDS: Automation.
Isolation and purification.
Nucleic acids.
Polymerase chain reaction.

Introduction

The efficient extraction of target nucleic acids from clinical samples and removal of polymerase chain reaction (PCR) inhibitors is a key step in molecular diagnostic testing. When performed manually, this can be a long process, prone to human error, variable performance and contamination. Hence, automated systems have been developed to improve performance, consistency, turnaround time and throughput capacity.

The bioMérieux NucliSENS easyMAG system (EM) is a low-throughput (one to 24 samples per run), semiautomated nucleic acid isolation (NAI) system. It has previously been validated for use in a range of diagnostic assays for viral pathogens,[1–5] and has been used routinely in the authors' laboratory for the past three years. However, this method proved inadequate for the increased workload observed during the H1N1 influenza pandemic in 2009. Therefore, a fully automated NAI robot, the Qiagen QIAsymphony SP (QS), was introduced to provide high-throughput capacity in detecting pandemic 2009 H1N1 influenza A.

Following the successful introduction of QS for influenza pandemic work, it became desirable to incorporate this system into the entire molecular diagnostics service. Further evaluation of QS for this purpose was achieved through processing of Quality Control for Molecular Diagnostics (QCMD) panels and through comparison with EM processing of pure virus preparations and various clinical specimens.

Materials and methods

Virus cultures and clinical specimens

Viruses (pure cultures and clinical specimens) used in this study included seasonal influenza A virus (H1N1 and H3N2), herpes simplex virus (HSV) 1 and 2, enterovirus, norovirus (NoV), rhinovirus (RV), hepatitis C virus (HCV), adenovirus, and cytomegalovirus (CMV).

Human influenza virus A/PuertoRico/8/34 (H1N1, Cambridge lineage; $1.4x10^8$ plaque-forming units [pfu]/mL) was grown in embryonated eggs and titrated in Madin Darby canine kidney cells (MDCK), as previously described.[6,7] Human influenza virus A/Brisbane/10/2007 (H3N2) was grown and titrated in MDCK cells.[8] Herpes simplex virus-1 (SC16; $1x10^9$ pfu/mL) was prepared as previously described.[9] Pure cultures of adenovirus and enterovirus, isolated from clinical diagnostic specimens in the Cambridge Health Protection Agency (HPA) diagnostic laboratory, were propagated in the PLC/PRF/5 primary liver carcinoma cell line, as previously described.[10] Virus stocks were prepared by freeze-thawing, centrifugation and recovery of the supernatant.

A total of 50 different clinical specimens, previously tested positive by routine real-time PCR assays in the Cambridge HPA diagnostic laboratory, were used in this study. These comprised 10 of each for rhinovirus (nasopharyngeal aspirates, tracheostomy aspirates, bronchoalveolar lavage and nose/throat swabs), norovirus (faeces), HCV (plasma), CMV (plasma), and HSV 1 and 2 (genital swabs). The residual positive samples were retrieved from the virology laboratory following routine processing.

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Table 1. Detection of QCMD 2010 influenza virus A and B RNA EQA programme panel samples.

Panel code	Matrix*	Sample contents†	Expected result	Sample type‡	QCMD Ct§	Influenza A Ct	H1 Ct	H1v Ct	H3 Ct	Influenza B Ct	2010 Ct#
INFRNA10-01	VTM	Influenza virus H3N2	Positive (InfA)	Core (InfA)	30	35			29		25
INFRNA10-02	VTM	Influenza virus H1N1v	Positive (InfA)	Core (InfA)	35	42		31			27
INFRNA10-03	VTM	Influenza virus H1N1	Positive (InfA)		33	35	31				28
INFRNA10-04	VTM	Influenza A/B-negative	Negative	Core							
INFRNA10-05	VTM	Influenza virus H1N1v	Positive (InfA)	Core (InfA)	35	44		33			26
INFRNA10-06	VTM	Influenza virus B (Victoria)	Positive (InfB)	Core (InfB)	32					26	25
INFRNA10-07	VTM	Influenza virus B (Yamagata)	Positive (InfB)	Core (InfB)	39					26	23
INFRNA10-08	VTM	Influenza virus H1N1v	Positive (InfA)	Core (InfA)	30	40		30			23
INFRNA10-09	VTM	Influenza virus H1N1	Positive (InfA)	Core (InfA)	29	31	28				25
INFRNA10-10	VTM	Influenza A/B-negative	Negative	Core							
INFRNA10-11	VTM	Influenza virus H1N1v	Positive (InfA)		38	49		42			29
INFRNA10-12	VTM	Influenza virus H3N2	Positive (InfA)		37	39			32		27

* VTM: virus transport medium.

† H1N1v: new-variant pandemic 2009 H1N1 strain.

‡ 'Core' refers to samples that must be correctly identified to comply with QCMD standards.

§ Ct values are for QCMD internal purposes only: they should not be used by participants for method comparison or as a target for individual laboratory assessment.

Result obtained after EM NAI as part of routine QCMD testing during 2010.

Personal details of all the samples selected for the study were anonymised to ensure confidentiality. Ethical approval was not required for this as it was considered to be a service evaluation using previously tested, anonymised samples.

Preparation of virus culture and clinical samples

Serial 10-fold dilutions of pure virus preparations were made in virus transport medium (VTM; Remel M4-RT, Fisher Scientific, Loughborough, UK) and used for NAI evaluations. Clinical faecal samples were treated with chloroform and centrifuged before NAI, as described previously.[11] Other clinical diagnostic samples were processed directly with no pretreatment. One positive specimen of each type (with high viral titre) was diluted 10-fold to a 10^{-5} concentration in phosphate-buffered saline and NAI was performed on each dilution. This allowed comparison of detection across a large range of viral concentrations.

Molecular test system

Viral nucleic acid was extracted using either the QIAsymphony virus/bacteria mini (200 µL; Cat. No. 931036) and midi (800 or 1000 µL; Cat. No. 931055) kits (Qiagen) on the QS instrument, employing the virus cell-free protocol or the generic 2.0.1 protocol (200, 500 or 1000 µL) with 'off board' lysis on the EM instrument. The latter was considered as the reference system.

Nucleic acid isolation and detection

Nucleic acid was extracted from 200–1000 μL of sample and eluted in 85 µL, of which 5 µL was used as template in 25-µL real-time PCR reactions. Influenza,[7,8] norovirus[11] and HCV[12] were detected using previously described real-time PCR assays. The other viral targets were detected using routine diagnostic assays (protocols available on request from MDC).

As an indication of nucleic acid extraction and PCR amplification efficacies, intact bacteriophage MS2 particles (4600 pfu) were added to RNA virus samples before automated NAI.[11] For DNA viruses, mouse CMV DNA (approximately 1000 copies per reaction) was added to real-time PCR reactions as a control for PCR inhibition only. These controls were detected in multiplex real-time PCR reactions with the target pathogen nucleic acids. Real-time PCR assays were conducted using the Rotor-Gene Q instrument (Qiagen), except for HSV QCMD samples, which were analysed using the LightCycler 2 (Roche).

Determination of test accuracies using QCMD materials

The accuracies of influenza and HSV detection by in-house real-time PCR assays, after QS NAI, were determined using the QCMD 2010 influenza virus A and B RNA external quality assessment (EQA) programme and the 2011 herpes simplex virus DNA EQA programme (QCMD, Glasgow, Scotland, UK) panels. Each panel consisted of samples that contained different concentrations of various viral species or serotypes, as well as negative samples. Panel details are shown in Tables 1 and 2. A sample volume of 200 µL was used for each panel, both of which had been tested previously by EM NAI using a 500 µL sample volume.

Evaluation of assay linearity

The linearity of influenza virus A (H1N1) and HSV-1 (SC16) detection by routine real-time PCR assay, after QS NAI, was determined using serial 10-fold dilutions of quantified viral preparations. The effect of sample volume on detection sensitivity was investigated using 200-µL and 1000-µL sample volumes. Extractions were performed in triplicate and each was tested by real-time PCR in triplicate.

Assay linearity was also evaluated using pure cultures of

influenza virus A/Brisbane/10/2007 (H3N2), adenovirus and enterovirus in qualitative assays. Single extractions from 200 µL (EM and QS), 500 µL (EM) and 800 µL (QS) sample volumes (according to current routine protocols) and single PCR tests were employed for these samples.

Evaluation of nucleic acid isolation

To evaluate the QS for use in routine clinical viral diagnostic assays, extractions were performed from anonymised remnant clinical specimens that previously tested positive following standard laboratory protocols employing NAI by EM and real-time PCR. These samples were stored at –20°C. Detection of viruses from 10-fold serial dilutions of high viral load clinical specimens was also used to give an indication of detection sensitivity and the dynamic range of the assay, and to identify whether or not PCR inhibitors were present. The lack of matrix effects in these dilution series was borne in mind while interpreting results. Sample volumes of 200 µL were used on both platforms.

Results

Test accuracies using QCMD materials

The QCMD 2010 influenza virus A and B RNA EQA programme panel consisted of 10 positive and two negative influenza samples (Table 1). The positives included four H1N1v, two H1N1, two H3N2 and two influenza B virus samples. The influenza A virus samples were provided as dilutions of one strain of each type in virus transport medium, and the two influenza B virus samples were two different strains.

After NAI by QS, all samples were identified correctly by real-time PCR assays (cycle threshold values shown in Table 1). Trends in cycle threshold (Ct) values, reflecting viral load of samples, were in concordance with those from QCMD for all H1N1 and H3N2 samples. For H1N1v samples, a two-cycle difference in Ct was observed between samples 2 and 5, which were stated to be duplicates. Otherwise, results for H1N1v reflected those from QCMD. The authors' assay gave equal Ct values for influenza B samples, whereas QCMD results showed a seven-cycle difference. The authors' results for the QCMD influenza panel, after NAI by QS, were also similar to those from 2010 after NAI by EM.

The 2011 herpes simplex virus DNA EQA programme panel consisted of eight positive and two negative HSV samples (Table 2). The positives included four HSV-1 and four HSV-2 samples, while the negatives included one varicella zoster virus-positive specimen. Both HSV samples were provided as dilutions of two different strains in virus transport medium.

After NAI by QS, all core samples were identified correctly by real-time PCR assays (Ct values shown in Table 2). However, two low-concentration samples were missed. Trends in Ct values, reflecting viral load, were in concordance with those from QCMD for all HSV samples. The authors' results for the QCMD HSV panel, after NAI by QS, were also similar to those after NAI by EM. However, after NAI by EM, only one of the low-level samples was missed (Table 2).

Evaluation of assay linearity

Table 3 shows Ct values for detection of RNA extracted from dilutions of human influenza virus A/PuertoRico/8/34 (H1N1, Cambridge lineage). Use of QS gave Ct values an average of 1.1 cycles (range: 0.2–1.6) lower than EM with a 200 µL sample volume, and 0.1 cycles (range: –0.4 to +0.5) higher than EM with a 1000 µL sample volume. Use of a 1000 µL sample volume with QS (QS1000) gave Ct values an average of 0.4 cycles (range: 0–1.1) lower than with a 200 µL (QS200) sample volume. Use of a 1000 µL sample volume with EM (EM1000) gave Ct values an average of 1.5 cycles (range: 0.7–2.2) lower than with a 200 µL (EM200) sample

Table 2. Detection of QCMD 2011 herpes simplex virus DNA EQA programme panel samples.

Panel code	Matrix*	Sample contents	Consensus sample concentration (copies/mL)†	Ct‡	Expected result	Sample type§	QS Ct (Type)	EM Ct (Type)
HSVDNA11-01	VTM	Herpes Simplex Virus (HSV-2, MS)	141	30	Positive		Neg	35.1 (2)
HSVDNA11-02	VTM	Herpes Simplex Virus (HSV-1, MacIntyre)	143	35	Positive		Neg	Neg
HSVDNA11-03	VTM	Herpes Simplex Virus (HSV-2, clinical)	50,350	33	Positive	Core	27.9 (2)	29.8 (2)
HSVDNA11-04	VTM	Herpes Simplex Virus (HSV-2, MS)	5875		Positive	Core	31.4 (2)	30.1 (2)
HSVDNA11-05	VTM	Herpes Simplex Virus (HSV-1, MacIntyre)	7396	35	Positive	Core	34.1 (1)	32.8 (1)
HSVDNA11-06	VTM	HSV Negative		32	Negative		Neg	Neg
HSVDNA11-07	VTM	Herpes Simplex Virus (HSV-2, clinical)	629,506	39	Positive	Core	24.7 (2)	22.9 (2)
HSVDNA11-08	VTM	Herpes Simplex Virus (HSV-1, 95/1906)	288	30	Positive		38.9 (1)	37.6 (1)
HSVDNA11-09	VTM	Varicella Zoster Virus		29	Negative	Core	Neg (VZV)	Neg (VZV)
HSVDNA11-10	VTM	Herpes Simplex Virus (HSV-1, 95/1906)	10,641		Positive	Core	33.4 (1)	32.1 (1)

* VTM: virus transport medium.

† Consensus values calculated from all of the data returned by participants, once outliers had been removed; the values are not technology specific and should not be used by participants for method comparison or as targets for individual laboratory assessment.

§ Ct values are for QCMD internal purposes only; they should not be used by participants for method comparison or as a target for individual laboratory assessment.

‡ 'Core' refers to samples that must be correctly identified to comply with QCMD standards.

Table 3. Detection of RNA from quantified pure influenza virus A (H1N1) culture.

Viral titre	EM200		EM1000		QS200		QS1000	
(pfu/mL)	Pos*	Ct (±2SD)	Pos	Ct (±2SD)	Pos	Ct (±2SD)	Pos	Ct (±2SD)
$1.4x10^5$	9/9	18.0 (±0.2)	9/9	16.5 (±0.8)	9/9	16.9 (±1.3)	9/9	16.9 (±0.2)
$1.4x10^4$	9/9	21.2 (±0.3)	9/9	20.5 (±0.3)	9/9	20.4 (±1.2)	9/9	20.3 (±0.3)
$1.4x10^3$	9/9	24.9 (±0.4)	9/9	23.9 (±0.4)	9/9	23.4 (±1.4)	9/9	23.6 (±0.4)
$1.4x10^2$	6/9	28.6 (±0.6)	9/9	26.8 (±0.4)	9/9	27.0 (±1.4)	9/9	27.2 (±0.5)
$1.4x10^1$	9/9	32.3 (±1.0)	9/9	30.1 (±0.7)	9/9	31.1 (±1.6)	9/9	30.5 (±0.7)
$1.4x10^0$	7/9	35.3 (±2.2)	9/9	33.5 (±1.2)	8/9	35.1 (±0.9)	9/9	34.0 (±1.6)

*Number of positive PCR results from nine replicate reactions.

volume. One replicate extraction failed for EM200 at the 1.4×10^2 dilution, but six out of six replicate PCR reactions were positive for the two successful extractions.

All four extraction protocols allowed detection of influenza virus over a concentration range of 1.40×10^5 to 1.40×10^1 pfu/mL in all replicate PCR reactions from successful extractions. At 1.40×10^0 pfu/mL, EM200 gave seven out of nine and QS200 gave eight out of nine positive PCR results, while all were positive by both EM1000 and QS1000. Average standard deviation for Ct values over the entire dilution range was 0.4 (EM200), 0.3 (EM1000), 0.7 (QS200) and 0.3 (QS1000).

Influenza A virus (H1N1) was detected over a 6 $\log_{10}$ concentration range with Ct values of 16.5–35.3. Linear regression analysis indicated linearity of the assay with the four NAI protocols over the concentration range (r^2 0.997–0.9999). Also, good correlation of results was observed between the four NAI (r^2 0.9952–0.9978).

Table 4 shows Ct values for the detection of DNA extracted from dilutions of HSV-1 (SC16). QS200 gave Ct values an average of 4.9 cycles (range: 3.8–8.5) higher than EM200, and QS1000 gave Ct values an average of 3.8 cycles (range: 0.4–7.1) higher than EM1000. QS1000 gave Ct values an average of 1.1 cycles (range: +0.6 to –3.5) lower than QS200, while EM1000 gave an equal average Ct value (range: –2.1 to +1.1) to EM200.

All four extraction protocols permitted detection of HSV-1 over a concentration range of 1.06×10^6 to 1.06×10^3 pfu/mL in all replicate PCR reactions from successful extractions. At 1.06×10^2 pfu/mL, EM200 gave eight out of nine and QS200 gave six out of nine positive PCR results, while all were positive by EM1000 and QS1000. At 1.06×10^1 pfu/mL, the number of positive replicates were nine/nine (EM200), seven/nine (EM1000), three/nine (QS200) and five/nine (QS1000). Average standard deviation for Ct values over the whole dilution range was 0.9 (EM200), 1.3 (EM1000), 2.0 (QS200) and 1.0 (QS1000).

HSV-1 (SC16) was detected over a 6 $\log_{10}$ concentration range with Ct values 15.6–43.6. Linear regression analysis indicated linearity of the assay with the four NAI protocols over the concentration range (r^2 0.951–0.99). Also, good correlation of results was observed between the four NAI protocols (r^2 0.9149–0.9908).

Nucleic acid isolation

Table 5 shows Ct values for detection of RNA and DNA extracted from human influenza virus A (H3N2), enterovirus and adenovirus.

Human influenza virus A (H3N2) was detected down to a 10^{-5} dilution with an 800 µL sample volume on QS (QS800), and down to a 10^{-6} dilution with QS200, EM200 and a 500 µL sample volume on EM (EM500). QS200 gave Ct values an average of 0.3 cycles (range: –0.2 to +0.9) higher than EM200, and QS800 gave an equal average Ct value (range: –0.2 to +0.4) to EM500. QS800 gave Ct values an average of 1.5 cycles (range: 1.2–1.8) lower than QS200, while EM500 gave Ct values an average of 1.8 cycles (range: +0.9 to –1.8) lower than EM200. Human influenza virus A (H3N2) was detected over a 5–6 $\log_{10}$ concentration range (Ct range: 15.8–34.0). Linear regression analysis indicated linearity of the assay with the four NAI protocols over the concentration range (r^2 0.9934–0.9982). Also, good correlation of results was observed between the four NAI protocols (r^2 0.992–0.9989).

Table 4. Detection of DNA from quantified pure herpes simplex virus 1 (SC16) culture.

Viral titre	EM200		EM1000		QS200		QS1000	
(pfu/mL)	Pos*	Ct (±2SD)	Pos	Ct (±2SD)	Pos	Ct (±2SD)	Pos	Ct (±2SD)
$1.06x10^6$	9/9	15.6 (±1.1)	9/9	16.6 (±1.3)	9/9	19.4 (±0.8)	9/9	19.6 (±0.2)
$1.06x10^5$	9/9	19.2 (±0.5)	9/9	19.3 (±0.8)	9/9	23.6 (±0.7)	9/9	23.1 (±0.4)
$1.06x10^4$	9/9	22.2 (±1.9)	9/9	22.7 (±0.8)	9/9	26.2 (±0.5)	9/9	26.8 (±0.8)
$1.06x10^3$	9/9	26.8 (±3.2)	9/9	26.2 (±1.9)	9/9	31.0 (±0.9)	9/9	30.6 (±1.5)
$1.06x10^2$	8/9	32.5 (±1.7)	9/9	33.6 (±7.9)	6/9	36.9 (±9.5)	9/9	34.0 (±3.2)
$1.06x10^1$	9/9	35.1 (±2.3)	7/9	33.0 (±3.1)	3/9	43.6 (±11.9)	5/9	40.1 (±5.5)

*Number of positive PCR results from nine replicate reactions.

Enterovirus was detected down to a 10^{-6} dilution with QS800, and down to a 10^{-7} dilution with QS200, EM200 and EM500. QS200 gave Ct values an average of 1.1 cycles (range: 0.2–2.0) higher than EM200, and QS800 gave Ct values an average of 1.1 cycles (range: 0.4–1.4) higher than EM500. QS800 gave Ct values an average of 1.4 cycles (range: 1.1–1.7) lower than QS200, while EM500 gave Ct values an average of 1.1 cycles (range: 0.2–1.6) lower than EM200. Enterovirus was detected over a 6–7 $\log_{10}$ concentration range (Ct range: 12.8–33.5). Linear regression analysis indicated linearity of the assay with the four NAI protocols over the concentration range (r^2 0.99–0.9997). Also, good correlation was observed between the four NAI protocols (r^2 0.9947–0.9989).

Adenovirus was detected down to a 10^{-7} dilution with all four NAI protocols. QS200 gave Ct values an average of 2.2 cycles (range: 0.8–4.6) higher than EM200, and QS800 gave Ct values an average of 1.4 cycles (range: 0.8–1.9) higher than EM500. QS800 gave Ct values an average of 1.5 cycles (range: 0–6.1) lower than QS200, while EM500 gave Ct values an average of 0.9 cycles (range +0.5 to –2.3) lower than EM200. Adenovirus was detected over a 7 $\log_{10}$ concentration range (Ct range: 12.3–40.2). Linear regression analysis indicated linearity of the assay with the four NAI protocols over the concentration range (r^2 0.9779–0.9955). Also, good correlation was observed between the four NAI protocols (r^2 0.9549–0.9972).

Clinical performance

All 50 previously positive clinical specimens were detected after NAI by both systems (Fig. 1). QS performed comparably to EM, giving average Ct differences of less than one cycle for four of the five targets (RV –0.7 [range –2.2 to 0]; NoV –0.3 [range –3.1 to +1.7]; CMV –0.6 [range –3.1 to +0.2]; HSV +0.3 [range –0.3 to +0.8]). For HCV, however, the average Ct difference was –2.6 (range –4.7 to –0.3). Linear regression analysis showed good correlation between the two methods for the same four targets (r^2 0.9316–0.984). For HCV, however, r^2 was 0.7907.

When high viral load samples were diluted 10-fold to a 10^{-5} concentration, linear regression analyses showed linearity for four of the five assays over the dilution range (r^2 0.989–0.9998). For CMV, however, r^2 was 0.9981 with QS and 0.9323 with EM. This lower r^2 value was due to one anomalously high Ct value for the most dilute sample detected. The r^2 value for the series without this anomalous value was 0.9992.

A similar pattern was observed for linearity of results between the two NAI methods, with three of the five targets giving r^2 0.9929–0.9984. Greater variability was again observed for CMV detection, with r^2 0.9404, which became 0.9987 without the most dilute sample. For HCV, QS again gave an average Ct value 1.6 cycles lower than EM (range –1.0 to –2.9), and linear regression analysis of QS results compared to EM results gave r^2 0.9863.

Inhibition of PCR reactions by nucleic acid extracts was not observed for any of the QCMD, pure culture or clinical specimens, as indicated by detection of target sequences and/or internal control sequences. The expected

Table 5. Nucleic acid isolation from serial dilutions of pure virus cultures of unknown concentration.

Virus	Dilution	EM200	QS200	EM500	QS800
Enterovirus	10^{-1}	14.4	14.6	12.8	13.2
	10^{-2}	17.7	18.6	16.2	16.9
	10^{-3}	20.9	22.2	19.7	21.1
	10^{-4}	24.1	25.6	22.8	24.0
	10^{-5}	27.5	28.8	26.2	27.6
	10^{-6}	29.8	31.8	29.2	30.5
	10^{-7}	32.8	33.5	32.6	ND
Adenovirus	10^{-1}	12.7	14.7	12.3	13.6
	10^{-2}	15.1	17.4	14.6	16.3
	10^{-3}	18.7	21.3	19.2	20.7
	10^{-4}	23.9	25.3	23.1	24.1
	10^{-5}	27.7	28.9	26.6	28.4
	10^{-6}	31.4	32.2	30.3	32.2
	10^{-7}	35.6	40.2	33.3	34.1
	10^{-8}	ND	ND	36.0	ND
Influenza virus A (H3N2)	10^{-1}	17.4	17.6	15.8	15.8
	10^{-2}	20.6	20.9	19.5	19.3
	10^{-3}	24.5	24.6	22.7	23.1
	10^{-4}	27.4	27.6	26.4	26.4
	10^{-5}	30.1	31.0	29.5	29.4
	10^{-6}	33.1	32.9	34.0	ND

Cycle threshold values are presented for detected virus samples. ND: not detected.

approximate 3.3 Ct increase between successive 10-fold dilutions of clinical extracts indicated that no PCR inhibitors were present.

Discussion

In this study, the fully automated, high-throughput QS system was evaluated for use in routine viral molecular diagnostic assays through QCMD testing, and by comparison to the laboratory standard, the EM system, for NAI from various DNA and RNA viruses and diverse clinical sample types.

Testing of QCMD panels is the benchmark for molecular diagnostic assays. Use of QCMD samples in this study allowed the authors to evaluate assay accuracy and challenge the system for detection of low viral load samples. For influenza virus detection, QS performed comparably to EM, with both extraction methods permitting detection and correct identification of all samples. However, EM extracts gave lower Ct values than QS extracts. This reflects the greater sample volume (500 µL) used for EM than for QS (200 µL). The EM NAI permitted detection of lower concentrations of HSV and gave lower Ct values than did QS NAI in all but one sample. Again, this reflects the larger sample volume used for EM extraction. However, both platforms permitted detection of all core samples from the QCMD panels used and, therefore, would be acceptable for use in influenza and HSV NAI.

Human influenza virus A/PuertoRico/8/34 (H1N1, Cambridge lineage) was detected from pure culture over a broad dynamic range of concentration (1.4×10^0 to 1.4×10^5 pfu/mL), with QS performing comparably to EM. A 1000 µL sample volume did not allow detection of higher dilutions of virus than did a 200 µL sample volume, but it did give more positive replicate detection results at lower viral loads. This would be expected as a larger sample volume will be more likely to contain virus particles at the level of 1.4 pfu/mL. Nonetheless, all assay formats demonstrated sensitive detection of influenza virus, suggesting applicability to clinical samples of low viral load.

Herpes simplex virus-1 (SC16) was also detected from pure culture over a broad dynamic range of concentration (1.06×10^1 to 1.06×10^6 pfu/mL), although the number of positive replicates decreased at the highest virus dilutions. The difference in observed assay sensitivity compared to influenza detection may be due in part to the particle:pfu ratio being higher for the influenza culture than for the HSV-1 culture, and to inherent inaccuracies in pfu determination. The EM consistently gave lower Ct values than QS and detected more positive replicates, suggesting its greater efficiency for HSV-1 DNA recovery. No difference in analytical sensitivity was observed between 200-µL and 1000-µL sample volumes. All assay formats demonstrated sensitive detection of HSV-1, again suggesting applicability to clinical samples even of low viral load.

As it is not possible to correlate clinically the causation or severity of viral upper respiratory and genital infection with viral load levels, minimum analytical sensitivities for these assays cannot be determined. Although a more sensitive assay would appear to be favourable, this may allow detection of transient carriage or dead virus not involved in disease causation. Clinical trials are required to evaluate

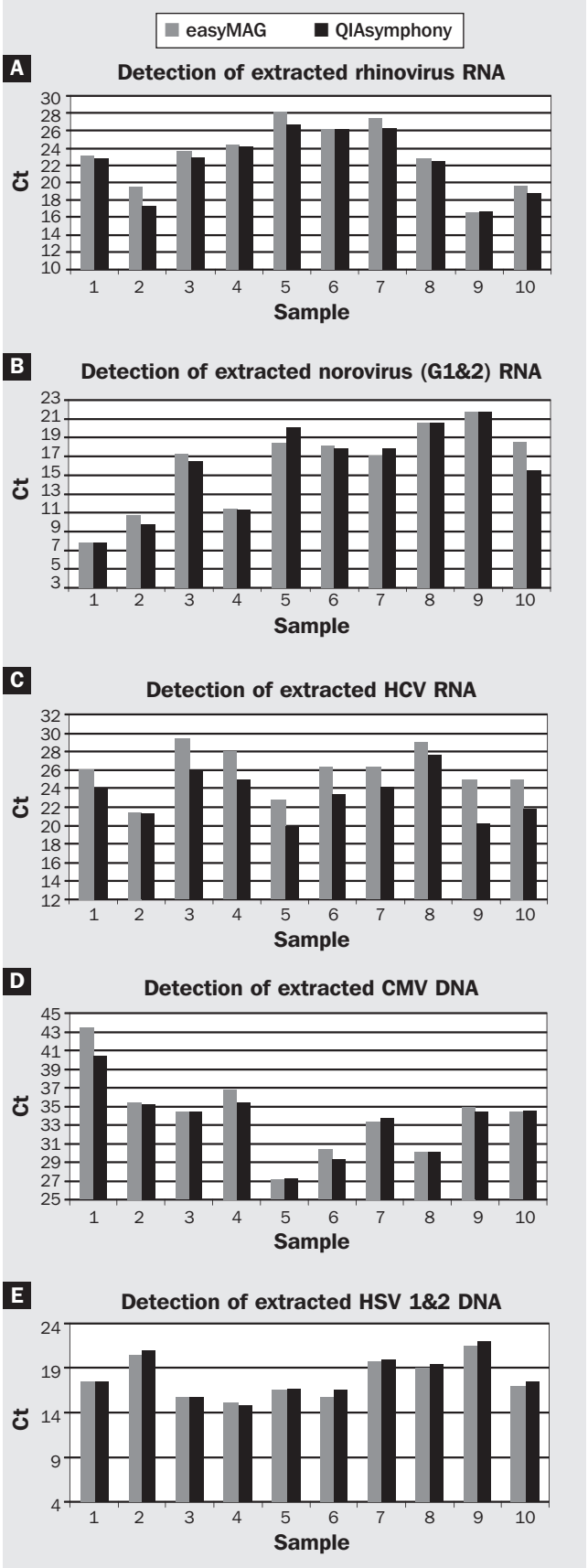

Fig. 1. Comparison of NAI methods for detection of viruses from clinical samples. Panels A–E show comparisons of Ct values for various viral pathogens after nucleic acid isolation from 10 individual clinical samples by easyMAG and QIAsymphony SP.

correlation between real-time PCR assay results and diagnosis of disease.

In further assay linearity experiments, influenza A virus (H3N2) was detected down to a 10^{-6} dilution of a pure culture, while adenovirus and enterovirus were detected down to a 10^{-7} dilution of pure culture. However, increasing the sample volume from 200 to 500 or 800 µL had variable effect, increasing or decreasing analytical sensitivity of the assay 10-fold, depending on the system and target. This variability may simply reflect the very low numbers of viral particles present at these high dilutions, as the probability of a sample containing virus particles reduces concomitantly. As such, variability in detection sensitivity may merely reflect sampling inefficiency. The lower Ct values produced using larger sample volumes was as expected and supports this theory. Similarly, Petrich *et al*.[13] found no difference in detection for severe acute respiratory syndrome (SARS) corona virus RNA from stool specimens when using 140- and 560-µL sample volumes with the QIAamp viral RNA kit. As such, it appears that increasing sample volume has a minimal effect on assay sensitivity.

Although these experiments were only performed from dilutions of viruses in buffer, the results should be equivalent to those for real clinical samples as no inhibitory effects on NAI or subsequent PCR are generally seen from respiratory samples in the authors' laboratory, which is demonstrated through successful amplification of internal controls. No titres were available for these cultures but the results suggest that the assays are sensitive and also demonstrate functionality over a broad dynamic range of viral load.

For downstream nucleic acid-based testing (e.g., PCR), inhibitors of enzymatic reactions must be removed during the NAI procedure. Substances such as proteins, carbohydrates and lipids can be removed by enzymatic treatment and precipitation with organic solvents. However, several studies have found that this is not sufficient to remove all PCR inhibitors from nucleic acid preparations obtained from various clinical samples.[14,15] Removal of PCR inhibitors has been achieved (with varying efficiencies) through the use of specialised buffers (e.g., STAR buffer, Roche), addition of PCR inhibitor binding matrices (e.g., InhibitEX, Qiagen; PrepMan Ultra, Applied Biosystems; acid-washed polyvinyl polypyrrolidone, Sigma), or through capture and washing of isolated nucleic acids on silica-based columns or beads.[14,16,17] Commercial kits are currently available that employ either silica-based columns or magnetic silica particles for NAI and purification, many of which have been automated for high throughput. However, not all kits are equally capable of removing PCR inhibitors from different sample types.[13,18–24] Thus, it is advisable to evaluate and optimise NAI methods for each sample type, and use of an internal control in PCR reactions is also important to monitor for PCR inhibition and for quality control.

Both NAI methods tested in this study permitted detection of viruses in all the clinical samples tested. Ct values were similar for four of the viral targets, but the QS system gave lower Ct levels for all the HCV samples tested. Further evaluation of NAI for HCV should be conducted to determine whether or not the choice of NAI system significantly affects detection and quantification.

Detection of viruses after five serial 10-fold dilutions of clinical specimens also demonstrated the sensitivity and broad dynamic range of the assays. Results of linear regression analysis indicated consistent efficiency of NAI across the range of virus concentrations, although the matrix effect was negated through dilution of samples in VTM. These results suggest that QS is comparable to EM for NAI from a range of RNA and DNA viruses present in diverse clinical sample types.

In this series of experiments, EM and QS performed comparably for detection of DNA and RNA viruses from pure cultures and diverse clinical specimens. This is not surprising because both automated systems use similar chemistries. Previous studies have found EM to be equivalent[2,3,5,25,26] or superior[18,20,27] to manual NAI methods for various viruses and bacteria. Moreover, EM has also been found superior to other automated NAI methods for various targets.[13,19,23,27] However, one study[24] found EM to be inferior to both the MagNA Pure system (Roche) and the BioRobot EZ1 (Qiagen) for *Toxoplasma* detection from amniotic fluid.

To date, few studies have investigated the efficiency of NAI with the QS. However, Miller *et al*.[28] showed that it compared favourably to the BioRobot EZ1 for CMV NAI from human serum, and Raggam *et al*. validated its use for CMV detection from whole blood[29] and also for EBV detection from EDTA-anticoagulated whole blood.[30] One recent study has shown that QS and EM produce comparable results for detection of enteric pathogens in faecal samples.[31]

The present study shows that the QS can extract viral nucleic acids from diverse clinical sample types and remove PCR inhibition sufficiently to allow real-time PCR amplification. Moreover, the system appears to be comparable to currently used methods. Thus, it would appear that these automated systems can be used interchangeably in the routine diagnostic laboratory.

Conclusions

The QS performs comparably to the authors' laboratory standard, the EM system, for NAI from DNA or RNA viruses in pure cultures and clinical samples. As such, it would be suitable for use in routine viral molecular diagnostics. Use of a 200 µL sample volume affords similar detection abilities to a 1000 µL sample volume and is preferred in order to minimise sample usage. With its high-throughput capacity, four times greater than available on the EM system, QS can provide surge capacity in peak seasons of virus infection and thus play an important role in centralised diagnostic laboratories. □

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