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Journal of Clinical Virology 35 (2006) 141–146

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QIAamp MinElute Virus kit effectively extracts viral nucleic acids from cerebrospinal fluids and nasopharyngeal swabs☆

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Received 10 March 2005; received in revised form 23 May 2005; accepted 25 May 2005

Abstract

Background: Nucleic acid preparation from a variety of clinical specimens requires efficient target recovery and amplification inhibitor removal and is critical for successful molecular diagnosis. The QIAamp MinElute Virus kit (Qiagen Inc., Valencia, CA) was compared to the two existing methods currently used in our laboratory, IsoQuick (Orca Research Inc., Bothell, WA) for DNA extraction and RNAzol B (Leedo Laboratories Inc., Houston, TX) for RNA extraction, of viral nucleic acids.
Study design: A total of 150 clinical specimens, including cerebrospinal fluid (CSF) and nasopharyngeal swabs (NPS), were used to determine the extraction efficiency of the MinElute compared to the other two methods. Nucleic acid recovery, hands-on time, turn-around-time and cost were compared across all kits.
Results: There was complete concordance between the MinElute and IsoQuick/RNAzol kits when herpes simplex virus (HSV), Epstein–Barr virus (EBV), varicella-zoster virus (VZV), influenza A virus or enteroviruses were detected using a colorimetric microtiter plate PCR system. The kits were equivalent in their ability to detect either DNA or RNA with superior ability to recover a high quality and quantity of RNA. With the potential to process larger specimen volumes, the MinElute kit can significantly shorten processing time from 2 h to 50–55 min.
Conclusions: Although relatively high test kit costs were noted, the MinElute kit provides another rapid and user-friendly specimen processing tool in the diagnostic molecular microbiology laboratory.

Keywords: Nucleic acid extraction; PCR; Herpesviruses; Enteroviruses; Influenza A virus

1. Introduction

In vitro nucleic acid amplification tests are now being incorporated more and more into clinical laboratories due to their high sensitivity and quick test turnaround time. One example is in the diagnosis of herpes simplex virus (HSV) encephalitis versus enteroviral meningitis. Central nervous system (CNS) infections caused by the two viruses can be difficult to distinguish clinically. Early treatment of HSV encephalitis can reduce patient morbidity and mortality (Skoldenberg et al., 1984; Whitley et al., 1986) while early diagnosis of CNS disease due to enterovirus can significantly shorten hospital stay and avoid overuse of broad range antibiotics (Ramers et al., 2000; Rotbart et al., 1994). On the other hand, early recognition of viral respiratory diseases caused by respiratory syncytial virus (RSV) and influenza viruses is critical for isolating patients to prevent nosocomial transmission (Rovida et al., 2005). Chemotherapy is more effective when the antiviral drugs are given at the earliest stage of diseases (Hayden et al., 1997; Treanor et al., 2000). For several viral diseases such as severe acute respiratory syndrome and

☆ This study was presented in part at the 20th Annual Meeting of the Pan American Society for Clinical Virology, Clearwater Beach, FL, USA, 25–28 April 2004.

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doi:10.1016/j.jcv.2005.05.011

aseptic meningitis, the viral loads in clinical specimens could be low (Lai et al., 2003; Peiris et al., 2003); therefore, a technique to process larger amounts of specimens to reach greater sensitivity is desirable.

There are rapid antigen tests available for both RSV and influenza, but these tests have low sensitivity (Boivin et al., 2004; Rovida et al., 2005). Molecular tests are now available that can be performed as an alternative to or in addition to these rapid tests, providing both a rapid and sensitive method to detect respiratory viruses. When using molecular techniques for virus detection, it is important to find a method for extracting nucleic acid from clinical samples that will concentrate the sample, provide a maximum yield of nucleic acids and also remove inhibitory substances. Improvements in nucleic acid extraction methodology have resulted in better target nucleic acid recovery and amplification inhibitor removal (Espy et al., 2001; Read, 2001). The more a target organism is present in a sample, the more chance there is of detecting the organism using PCR. If a larger specimen volume is processed, then more nucleic acid can be isolated. A nucleic acid extraction system that can handle diversified clinical specimens with different volumes is desirable.

We have been using IsoQuick and RNAzol B for DNA and RNA extraction in our clinical diagnostic services (Smalling et al., 2002; Tang et al., 1998, 1999). Both kits work well in concentrating and isolating nucleic acids while removing inhibitors; however, they are time-consuming procedures, and cannot be adapted to process larger volumes of sample. Recently, a QIAamp MinElute kit (Qiagen Inc., Valencia, CA) became available, which can be used for simultaneous purification of viral RNA and DNA from a variety of specimen types without organic extraction or alcohol precipitation. A specimen can be processed by using either a vacuum or a spin procedure. With an additional adaptor, the MinElute system can process specimens with large and variable volumes. In this study, we have chosen both nasopharyngeal swab (NPS) and cerebrospinal fluid (CSF) specimens submitted for influenza A virus, enterovirus and herpesvirus testing to validate the MinElute nucleic acid extraction system. The quantity and quality of the extracted nucleic acids and the sensitivity and reproducibility for relevant pathogen detection were compared to the currently used IsoQuick/RNAzol B methods. In addition, total extraction time and technologist hands-on time as well as costs for performing the system were determined.

2. Materials and methods

2.1. Clinical specimens

A total of 150 clinical specimens were included in the evaluation. Forty-nine CSF samples were collected from patients with suspected viral CNS disease who visited Vanderbilt University Medical Center between April 2002 and November 2003. An aliquot of CSF was frozen at −70 °C until tested by both extraction methods. NPS specimens were collected through a CDC-sponsored New Vaccine Surveillance Network between October 2002 and March 2003. Specimens collected by two Dacron swabs were immediately placed in 2 ml of Hanks' viral transport medium, and 0.2 ml of specimen suspension was mixed with 0.9 ml of a guanidine thiocyanate lysis buffer (NucliSens, bioMerieux, Durham, NC). The sample mixture was incubated for 10 min and then frozen at −80 °C until tested.

2.2. DNA extraction

For the IsoQuick protocol, 100 μl of CSF were extracted, and viral DNA was resuspended in 25 μl of RNase-free water as previously published (Smalling et al., 2002; Tang et al., 1998). For the MinElute protocol, viral DNA was extracted by the MinElute spin kit according to the manufacturer's instructions. In brief, 25 μl of protease was added to 100 μl of CSF; a Buffer AL/Carrier RNA mixture was made and added to the CSF/protease mixture. The sample mixture was incubated at 56 °C for 15 min, and 250 μl ethanol was added. The entire volume of sample was then transferred to a QIAamp MinElute column and centrifuged for 1 min at 6000 × *g*. A series of washes were performed and the total viral DNA was eluted into 55 μl of RNase-free water.

2.3. RNA extraction

For the RNAzol B protocol, 100 μl of CSF or NPS was extracted as previously published (Smalling et al., 2002; Tang et al., 1999). For the MinElute protocol, viral RNA was extracted by the MinElute Vacuum kit according to the manufacturer's instructions. In brief, 100 μl CSF or 1.1 ml NPS-lysis buffer mixture was mixed with 75 μl of protease, and then 550 μl of a Buffer AL/Carrier RNA mixture was added. After incubating at 56 °C for 15 min, 600 μl of ethanol was added and the mixture was incubated for five more minutes. The entire volume of sample was then added to a spin column connected to a vacuum manifold. The sample was then drawn through the column to allow the nucleic acids to bind to the silica membrane. A series of washes were performed and viral RNA was eluted into 55 μl of sample diluent (Applied Biosystems, Branchburg, NJ) by centrifuging for 1 min at 12,000 × *g*.

2.4. PCR–EIA

A colorimetric microtiter plate PCR (PCR–EIA) system was used for the detection of herpesviral DNA, which included HSV, varicella-zoster virus (VZV) or Epstein–Barr virus (EBV) in CSF as previously described (Tang et al., 1997, 1998). DNA extraction volumes added into the PCR reaction were 10 μl from IsoQuick and 20 μl from MinElute. All extracted samples were also tested for the β-actin "housekeeping" gene to detect the ability of the extraction kits to remove inhibitors (Li et al., 2003). Output signals were

measured at optical densities of 450 nm (OD_{450}) and 490 nm (OD_{490}). A positive result was defined as an $OD_{450} - OD_{490}$ value greater then or equal to 0.1 (Tang et al., 1998).

2.5. RT-PCR–EIA

A similar RT-PCR–EIA system was used for the detection of all RNA targets, including enteroviruses (CSF) and influenza A virus (NPS), as described previously (Smalling et al., 2002; Tang et al., 1999). Twenty-five microliters of extracted RNA by RNAzol B or MinElute was added into the RT-PCR reaction. The primers and probes used for influenza A virus amplification and identification were described previously (Poehling et al., 2002). All extracts also were tested using a β-actin housekeeping gene with the same protocol by incorporating β-actin primers (forward: 5′-TTT CGT GGA TGC CAC AGG ACT-3′; antisense: 5′-TGG CCA CGG CTG CTT CCA GCT-3′) and probe (5′-CTC TTC CAG CCT TCC TTC CTG-3′). Output signals were measured at optical densities of 450 nm (OD_{450}) and 490 nm (OD_{490}). A positive result was defined as an $OD_{450} - OD_{490}$ value greater then or equal to 0.1 (Tang et al., 1999).

2.6. DNA quantitation

A real-time TaqMan PCR assay was used to compare the DNA recovery of the IsoQuick and MinElute extraction kits. A standard curve was achieved by using serial dilutions of a plasmid standard containing the primer-spanning region of the CMV glycoprotein B gene (Li et al., 2003). CMV DNA amplification was performed in a "real-time" format on the 7700 ABI Prism Sequence Detector (Applied Biosystems, Foster City, CA). One CSF specimens was spiked with a CMV plasmid to make a final concentration of 2.69×10^4 copies/reaction and extracted by using both the IsoQuick and MinElute. CMV quantitation of the DNA extracts were performed on the 7700 ABI Prism Sequence Detector as previously described (Li et al., 2003).

2.7. RNA quantitation

A real-time TaqMan RT-PCR assay was used to compare DNA recovery of the IsoQuick and MinElute extraction kits. A standard curve was achieved by using serial dilutions of HIV-1 RNA extracted from an HIV-1-positive plasma with a known viral load. Three CSF specimens were spiked with an HIV-1-positive plasma to make a final concentration of 2.97×10^7 copies/reaction and extracted by using both the RNAzol B and MinElute. HIV-1 RNA amplification was performed on the 7700 ABI Prism Sequence Detector. A 25 μl reaction mixture containing 10 μl of total RNA, 0.5 μM of each primer and 0.2 μM of TaqMan probe was mixed with 25 μl of the TaqMan One-Step RT-PCR 2× Master Mix (Applied Biosystems). The reaction conditions were designed as follows: RT at 48 °C for 30 min and initial denaturation at 95 °C for 10 min followed by 40 cycles with 15 s at 95 °C for denaturing and 1 min at 60 °C for annealing and extension (Deng et al., 2003). A primer set for HIV-1 (gagF578: 5′-AYC ARG CAG CYA TGC AAA TGT T-3′ and gagR730: 5′-CTG AAG GGT ACT AGT AGT TCC TGC TAT RTC-3′, Y = C or T, R = A or G) was designed to amplify a 152 base pair fragment in the HIV-1 *gag* gene (Kwok et al., 1987). The probe (gagP607: 5′-ACC ATC AAT GAG GAA GCT GCA GAA TGG GA-3′) was dually labeled with a reporter dye (FAM, 6-carboxy fluorescein) at the 5′ end and a quencher dye (TAMRA, 6-carboxytetramethyrhodamine) at the 3′ end.

2.8. Cost, total extraction time and hands-on time analysis

The cost per test was calculated for each assay and included test kit, materials and reagents. Laboratory personnel salaries, equipment and laboratory overhead costs were not included in this cost. Both hands-on time and total extraction time were counted when incubation or centrifuge time was equal to or less than 5 min while only total extraction time was counted when incubation or centrifuge time was greater than 5 min. An additional 15–20% turn-around-time was added to each procedure to account for specimen processing, reagent preparation, bench cleaning and record keeping. A cost saving due to shortened labor was estimated for each procedure based on a batch size of 10.

3. Results

A total of 150 clinical samples were included in this study to validate the MinElute kit in comparison to either the IsoQuick (DNA detection) or RNAzol B (RNA detection) kit.

Table 1
Virus detection concordance in nucleic acids extracted by different extraction kits

Specimen/virus	No. tested	RNAzol or IsoQuick+ MinElute+	RNAzol or IsoQuick+ MinElute−	RNAzol or IsoQuick− MinElute+	RNAzol or IsoQuick− MinElute−	Percentage matched
CSF/enterovirus	20	7	0	0	13	100
CSF/HSV	20	6	0	0	14	100
CSF/EBV	7	6	0	0	1	100
CSF/VZV	2	2	0	0	0	100
NPS/influenza A	101	36	0	1	64	99.0
Total	150	57	0	1	92	99.3

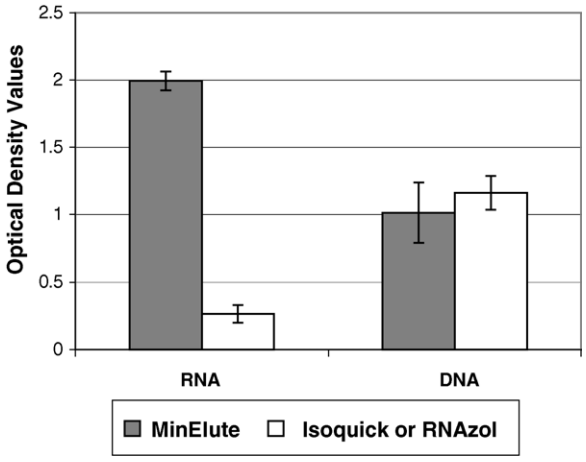

Fig. 1. Optical density value comparison between the MinElute Spin kit and IsoQuick for DNA extraction and the MinElute Vacuum kit and RNAzol B for RNA extraction.

Human β-actin DNA or RNA was detected in all nucleic acid specimens extracted by either MinElute or IsoQuick/RNAzol B kit, indicating these nucleic acid extracts were free of amplification inhibitors.

All 101 NPS RNA specimens, which were extracted by the MinElute and RNAzol B, were tested for influenza A virus by RT-PCR–EIA. There was a 99% agreement between the MinElute and RNAzol B when influenza A virus was detected; one was detected in the MinElute but not the RNAzol B-extracted RNA (Table 1). Twenty CSF samples were extracted by using the MinElute and RNAzol B in parallel, and tested for enteroviruses by RT-PCR–EIA. There was 100% agreement among all 20 samples between the two extraction methods (Table 1). Twenty-nine CSF samples were extracted by using the MinElute and IsoQuick in parallel, and tested for HSV, EBV or VZV by PCR–EIA. There was 100% agreement again between the two extraction methods for herpesvirus detection. These results suggested that the MinElute possesses a similar sensitivity as with the IsoQuick/RNAzol B for both DNA and RNA virus detection.

An analytical sensitivity of extraction kits was evaluated by extracting 10 CSF samples in parallel using the MinElute and IsoQuick/RNAzol B. Nucleic acid extracts were serially diluted and tested for RNA (enterovirus and influenza A virus) and DNA (HSV, EBV and VZV) by RT-PCR–EIA and PCR–EIA (Fig. 1). These extractions were tested at concentrations of undiluted, 1:10, 1:100 and 1:1000. When RNA extraction methods (MinElute versus RNAzol B) were compared, the MinElute produced significantly larger optical density (OD) values than the RNAzol B ($p < 0.001$). Samples extracted by the MinElute tested positive to the 1:1000 dilution while those extracted by the RNAzol were only positive in the undiluted concentrations. The OD values were similar when DNA extraction methods were compared between the MinElute and IsoQuick ($p > 0.05$). Both extraction methods produced positive results to the 1:1000 dilutions. These data indicated that while both IsoQuick and MinElute yielded similar amounts of DNA, the MinElute might yield higher quantities of RNA than RNAzol.

Table 2
Nucleic acid recovery rate contrast between the IsoQuick/RNAzol B and MinElute kits

Organism (copies/reaction)	IsoQuick or RNAzol (%)	MinElute (%)
HIV-1 (2.97×10^7)	$[4.5 \pm 1.7] \times 10^6$ (15.2)[a]	$[6.0 \pm 2.9] \times 10^6$ (20.3)
CMV (2.69×10^4)	$[1.8 \pm 0.2] \times 10^4$ (66.9)	$[2.0 \pm 0.2] \times 10^4$ (74.3)

[a] Mean ± S.D. (recovery percentage) from triplicate runs. There were no statistical differences in recovery rates between MinElute and IsoQuick/RNAzol B for both HIV-1 and CMV quantitation ($p > 0.05$).

Nucleic acid recovery rates between the MinElute and RNAzol B were further determined by quantitating HIV-1 or CMV viral loads in extracted RNA or DNA specimens using a real-time TaqMan PCR. The samples were run in triplicate along with an unextracted control sample and the recovery rate was calculated by comparing the viral loads in the control. The MinElute and IsoQuick had recovery rates for CMV DNA of 74.3% and 66.9% and the MinElute and RNAzol B had recovery rates for HIV-1 RNA of 20.3% and 15.2% (Table 2). While the DNA recovery rate was higher than the RNA for both extraction methods, there were no statistical differences in recovery rates between MinElute and IsoQuick/RNAzol B for both HIV-1 and CMV quantitation ($p > 0.05$). These data indicated that MinElute possesses a similar organism-specific nucleic acid recovery rate in comparison to the IsoQuick for DNA and the RNAzol B for RNA.

Cost per test was calculated for each extraction kit, which included test kit and additional materials and reagents used for nucleic acid extraction. The cost per test was US$ 2.86 for the IsoQuick, which was cheaper than the costs of the

Table 3
Summary of test costs, hands-on time and total extraction time among the extraction kits

Parameters	IsoQuick	MinElute Spin	RNAzol B	MinElute Vacuum
Costs/test (US$)	2.86	4.69	4.42	4.54
Labor cost saved per test[a] (US$)	Not applicable	0.70	Not applicable	2.50
Hands-on time (min)[b]	46.1 ± 4.8[c]	30.4 ± 2.6	36.2 ± 4.4	36.1 ± 3.0
Total extraction time (min)[b]	124.5 ± 6.6	49.0 ± 3.5	122.1 ± 8.2	54.6 ± 3.9

[a] Based on a batch size of 10 samples and an hourly wage of US$ 20.00.
[b] Hands-on time and total extraction time were estimated from four full runs with two technologists run on two different days.
[c] Mean ± S.D. There was no statistical significance in hands-on time among the kits. Total extraction time for IsoQuick and/or RNAzol B was significantly longer than the MinElute.

RNAzol B (US$ 4.42), MinElute Vacuum (US$ 4.54) and MinElute Spin (US$ 4.69). The MinElute Spin kit had the lowest hands-on time at 30 min and the IsoQuick had the highest hands-on time at 45 min in comparison to 35 min for the RNAzol B and MinElute Vacuum kits. The total time for DNA/RNA purification for the MinElute Vacuum and Spin kits were 50–55 min, which were about half of the time needed for the IsoQuick and RNAzol B kits. The labor cost savings of the MinElute kit were US$ 2.50 and 0.70 per test for RNA and DNA extraction, respectively (Table 3).

4. Discussion

The purpose of this study was to validate the MinElute Vacuum and Spin kits against our currently used IsoQuick and RNAzol B kits. Both MinElute kits were able to effectively remove the amplification inhibitors existing in NPS and CSF specimens. The MinElute kits possessed an equivalent sensitivity in nucleic acid recovery and detection, in comparison to the IsoQuick and RNAzol B, for both DNA and RNA extraction. With adaption, the MinElute can process variable and larger volumes to enhance test sensitivity which is important when the viral loads are extremely small (Lai et al., 2003; Peiris et al., 2003). On the other hand, the MinElute shortened the total time for DNA/RNA purification from 2 h to less than 1 h, which provided another rapid and user-friendly specimen processing tool in the diagnostic molecular microbiology laboratory.

Good specimen preparation and extraction comprises efficient target recovery, establishment of the integrity of nucleic acid targets, optimal removal of amplification inhibitors, elimination of components that affect other enzymatic substrates and sterilization of potentially hazardous organisms. The Qiagen MinElute kit was designed for simultaneous purification of viral RNA and DNA from plasma, serum and cell-free body fluids using vacuum or spin processing. Nucleic acids bind specifically to the silica-gel membrane while contaminants pass through (Boom et al., 1990). No organic extraction or alcohol precipitation was involved in the processing, which is superior to phenol/chloroform-based chaotropic lysis included in the IsoQuick and RNAzol B kits (Smalling et al., 2002; Tang et al., 1998, 1999). Several components in the organic phase (e.g., phenol) are strong amplification inhibitors. During specimen processing, if residual organic phase was carried over, the phenol would bind and inactivate the DNA polymerase and cause false negative results (Katcher and Schwartz, 1994). To avoid residual phenol carryover, we have implemented an additional spin step to make sure all organic solvents are removed. This, in turn, compromises DNA recovery due to loss of some of the aqueous phase and increases the time needed for specimen processing.

The MinElute simplified and hastened viral RNA and DNA isolation processing. The MinElute improved patient care through significant shortening of test turn-around-time, which is extremely attractive in the clinical setting when test results determine the urgent clinical intervention (Hayden et al., 1997; Ramers et al., 2000). The high cost for performing the MinElute was noted. Since laboratory labor was not calculated in the cost per test, it is acceptable to assume that the savings in hands-on time and turn-around-time for performing the MinElute would offset the higher costs of the test kit.

5. Conclusions

Both MinElute Vacuum and Spin kits provided rapid purification of high quality viral DNA and RNA. The system yielded highly purified nucleic acids, which were free of contaminants and inhibitors, as well as an improved test turn-around-time by approximately 1 h. Although relatively high test kit costs were noted, the MinElute kit provides another rapid and user-friendly specimen processing tool in the diagnostic molecular microbiology laboratory.

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