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

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**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

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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