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Automated, simple, and efficient influenza RNA extraction from clinical respiratory swabs using TruTip and epMotion

Sara B. Griesemer[a,*], Rebecca Holmberg[b], Christopher G. Cooney[b], Nitu Thakore[b], Alissa Gindlesperger[b], Christopher Knickerbocker[b], Darrell P. Chandler[b], Kirsten St. George[a]

[a] Laboratory of Viral Diseases, Wadsworth Center, New York State Department of Health, 120 New Scotland Avenue, Albany, NY 12208, United States
[b] Akonni Biosystems Inc., 400 Sagner Avenue, Suite 300, Frederick, MD 21701, United States

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ABSTRACT

Background: Rapid, simple and efficient influenza RNA purification from clinical samples is essential for sensitive molecular detection of influenza infection. Automation of the TruTip extraction method can increase sample throughput while maintaining performance.
Objectives: To automate TruTip influenza RNA extraction using an Eppendorf epMotion robotic liquid handler, and to compare its performance to the bioMerieux easyMAG and Qiagen QIAcube instruments.
Study design: Extraction efficacy and reproducibility of the automated TruTip/epMotion protocol was assessed from influenza-negative respiratory samples spiked with influenza A and B viruses. Clinical extraction performance from 170 influenza A and B-positive respiratory swabs was also evaluated and compared using influenza A and B real-time RT-PCR assays.
Results: TruTip/epMotion extraction efficacy was 100% in influenza virus-spiked samples with at least 745 influenza A and 370 influenza B input gene copies per extraction, and exhibited high reproducibility over four $\log_{10}$ concentrations of virus (<1% CV). RNA yields between the three automated methods differed by less than 0.5 $\log_{10}$ gene copies. 99% of clinical specimens that were PCR-positive after easyMAG or QIAcube extraction were also positive following TruTip extraction. Overall C_t value differences obtained between TruTip/epMotion and easyMAG/QIAcube clinical extracts ranged from 1.24 to 1.91. Pairwise comparisons of C_t values showed a high correlation of the TruTip/epMotion protocol to the other methods ($R^2 > 0.90$).
Conclusion: The automated TruTip/epMotion protocol is a simple and rapid extraction method that reproducibly purifies influenza RNA from respiratory swabs, with comparable efficacy and efficiency to both the easyMAG and QIAcube instruments.

1. Background

Influenza viruses continue to be a global public health threat, causing epidemics resulting in millions of clinical cases and up to 500,000 deaths each year [1,2]. Although influenza seasons vary in severity, approximately 5–20% of people are infected with influenza viruses annually in the United States [3], with an estimated 200,000 hospitalizations and up to 49,000 deaths each season [4,5]. Following the 2009 H1N1 pandemic, influenza testing demands significantly increased, reinforcing the need for simple, rapid, sensitive, and increased throughput influenza testing capabilities by diagnostic laboratories [6].

Due to the high sensitivity and specificity of molecular detection techniques, increasing numbers of diagnostic laboratories have implemented them to detect and characterize influenza viruses. High quality viral RNA is required for such techniques, and isolation of viral RNA from respiratory samples can be one of the most time-consuming steps, creating workflow bottlenecks in the diagnostic process. Several commercially available, automated RNA sample preparation systems exist but can be expensive, involve tedious or confusing instrument setup, require large quantities of consumables, have large footprints, or are inefficient at RNA extraction [7–11]. We recently described a simple, rapid, manual extraction method for purifying influenza RNA from respiratory samples [12]. Known as TruTip extraction, the process involves the

Abbreviations: WC-LVD, Wadsworth Center Laboratory of Viral Diseases; pRhMK, primary rheusus monkey kidney; NPS, nasopharyngeal swab; RT-PCR, reverse transcriptase-polymerase chain reaction; CDC, Centers for Disease Control and Prevention; ABI, Applied Biosystems; EPM, epMotion; SPT/LPT, small/large pore tips; SD, standard deviation; CV, coefficients of variation; C_t, crossing threshold.

* Corresponding author. Tel.: +1 518 474 4177; fax: +1 518 486 7971.
E-mail address: sbg03@health.state.ny.us (S.B. Griesemer).

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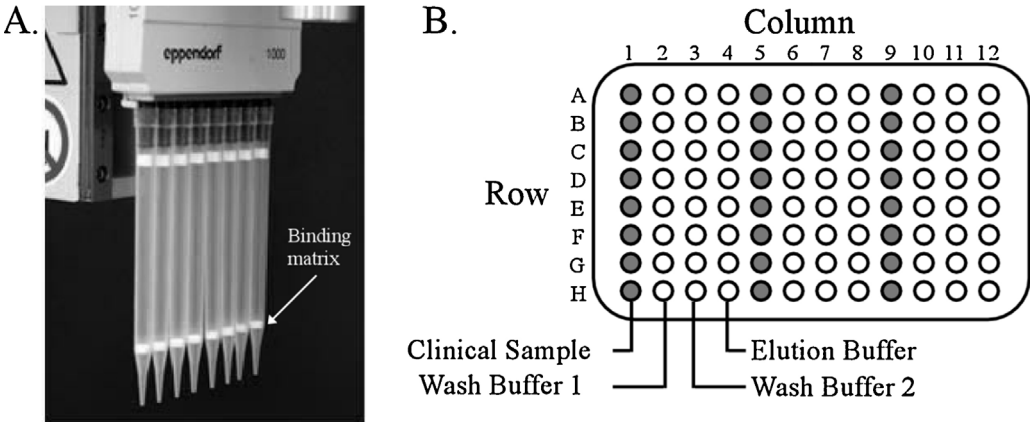

Fig. 1. (A) EPM TruTips on an epMotion 8-channel tool. (B) 96-well plate layout for extraction of 24 samples with the automated epMotion protocol. Lysed clinical samples are manually added to columns 1, 5, and 9. TruTip solutions are added automatically to the remaining wells by the epMotion script.

use of a nucleic acid extraction matrix embedded inside a pipette tip [13]. Here we developed and evaluated an automated TruTip extraction method using a robotic liquid handler, which improves sample throughput without sacrificing influenza RNA extraction and detection efficacy.

2. Objectives

To evaluate an automated TruTip RNA extraction procedure developed for respiratory samples using an epMotion 5070 liquid handling system (Eppendorf, Hauppauge, NY), and to evaluate the performance of the system relative to other automated extraction instruments commonly used in diagnostic laboratories (easyMAG and QIAcube).

3. Study design

3.1. Viral culture

Cultured viruses used for spike-recovery experiments included influenza A/New York/1669/2009(H1N1) and an influenza B virus isolated in 2008. Both viruses were isolated from respiratory samples submitted to the reference and surveillance programs at the Wadsworth Center Laboratory of Viral Diseases (WC-LVD), New York State Department of Health (Albany, NY). Viruses were propagated in primary rhesus monkey kidney (pRhMK) cells (Diagnostic Hybrids Inc., Athens, OH) using classical virus culture procedures.

To determine stock viral RNA concentrations, viral RNA was extracted from 60 μL of culture harvests and eluted into 60 μL using the QIAamp Viral RNA kit and QIAcube instrument (Qiagen, Valencia, CA) as per the manufacturer's instructions. Using the real-time RT-PCR assays described below, influenza RNA was quantified against standard curves created by serial dilutions of gene-specific influenza A and B RNA transcripts, developed in-house and previously quantified by UV spectrophotometry.

3.2. Virus-spiked samples

Influenza-negative nasopharyngeal swab specimens (NPS) in viral transport medium were pooled and used as sample matrix for epMotion protocol development, and for comparative evaluation of the easyMAG (bioMerieux, Durham, NC) and QIAcube extraction instruments. Quantified influenza A and B viruses were serially ($\log_{10}$) diluted in sample matrix from 2.98×10^7 to 298 RNA gene copies/mL (influenza A) and 1.48×10^7 to 148 gene copies/mL (influenza B). Spiked samples were dispensed into single-use aliquots and frozen at −70 °C. Quantitative real-time RT-PCR was performed on RNA extracts in duplicate.

3.3. Clinical specimens

In total, 170 influenza A or B positive respiratory swabs (nasopharyngeal, oropharyngeal, nasal, and throat) were randomly chosen from the 2008 to 2011 respiratory specimen archives of the WC-LVD. Samples were originally extracted using an easyMAG instrument, confirmed positive with the CDC Human Influenza Virus Real-time RT-PCR Diagnostic Panel [14], and stored at −70 °C. Influenza A subtypes included both A(H3N2) and A(H1N1)pdm09 viruses.

3.4. Real-time RT-PCR

Testing for influenza A and B RNA was performed using laboratory-developed influenza A and B real-time RT-PCR assays as previously described [15], using either Quanta BioSciences's qScript™ One-Step qRT-PCR (Gaithersburg, MD) kit with a Stratagene Mx3005P thermal cycler (Agilent Technologies, Santa Clara, CA), or an ABI TaqMan® Fast Virus 1-step Master Mix kit (Applied Biosystems, Foster City, CA) with an ABI 7500 Fast Dx real-time PCR instrument.

3.5. TruTip/epMotion RNA purification from virus-spiked samples

Automated TruTip RNA extraction was performed on an Eppendorf epMotion 5070 using a modified baseline protocol as recently described [16]. Briefly, an 8-channel pipetting tool was used with 1 mL epMotion (EPM) TruTips containing a small pore (SPT), 2 mm thick TruTip binding matrix (Fig. 1A). Off-board lysis was performed on virus-spiked NPS (250 μL sample:375 μL TruTip lysis buffer) followed by a 10 min incubation at room temperature. Lysed samples were manually transferred to a 2 mL 96-well deep-well sample plate, and the plate positioned on the epMotion worktable. After manual addition of reagents to bulk reservoirs the automated script was started. The epMotion first added reagents to the appropriate columns on the 96-well extraction plate (Fig. 1B). Next, the machine added 375 μL of 95% ethanol to the lysed samples and mixed each thoroughly by repeated pipetting. Extraction by the epMotion continued by aspirating and dispensing the lysed samples and wash buffers through the TruTip binding matrix, 8 samples at a time. Purified RNA was then eluted into the extraction plate by cycling 100 μL RNase-free Tris–HCl elution buffer through the TruTip binding matrix.