APPLICATION NOTE

Automated Workflow of Viral RNA/DNA Purification from Patient Samples on the Hamilton Microlab® STAR™ Liquid Handling System.

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Abstract

To reduce hands-on time and increase precision during SARS-CoV-2 RNA extraction BioEcho, together with Hamilton and the German diagnostics laboratory in Hamburg 'Labor Dr. Fenner und Kollegen', developed an automated method for the CE-IVD EchoLUTION Viral RNA/DNA Swab Kit. Here we established a protocol compatible with dry swabs and swabs in transport media in the Hamilton Microlab STAR Liquid Handling Systems. Our results showed that the automated method from BioEcho allows you to extract 4 x 96 samples, including PCR setup, in one hour 30 minutes after swab removal. The EchoLUTION Viral RNA/DNA Swab Kit automated protocol generates reliable, reproducible, and traceable results while increasing your throughput.

Introduction

The SARS-CoV-2 global outbreak generated a need for fast and reliable large-scale testing to diagnose COVID-19 and help prevent further transmission. Due to the accelerating emergence of new pathogenic variants, diagnostic laboratories focused their energy on molecular techniques like RT-PCR for viral detection. However, one of the main bottlenecks of the PCR test was the laborious and time-consuming molecular method for viral genetic material extraction. It was here that innovative technologies, like the EchoLUTION single-step extraction method from BioEcho, started to play an important role.

To meet the needs of diagnostic laboratories, BioEcho developed the EchoLUTION Viral RNA/DNA Swab Kit. The CE-IVD marked product allows the extraction of viral RNA and DNA and PCR setup from 4 × 96 samples in one hour 45 minutes after swab removal, when done manually. Moreover, to reduce hands-on time to a

minimum while increasing precision, BioEcho partnered with Hamilton to work on automated solutions.

BioEcho, together with Hamilton and the German diagnostics laboratory in Hamburg, 'Labor Dr. Fenner und Kollegen', developed the automated workflow for the 96- and 48-well formats of the CE-IVD EchoLUTION Viral RNA/DNA Swab Kit. This application note demonstrates the advantages of this product for diagnostics laboratories in processing patient samples, and the additional flexibility it provides to the workflow when used with liquid handling systems from Hamilton's Microlab STAR line.

The EchoLUTION technology

The EchoLUTION technology from BioEcho relies on single-step nucleic acid isolation, different from the *bind-wash-elute* nucleic acid extraction methods available on the market. This new technology retains impurities such as cellular debris and allows the DNA and RNA to pass through the matrix without further interactions. Additionally, the BioEcho products do not use organic solvents such as ethanol, avoiding DNA and RNA denaturation. Nucleic acids are isolated faster and free of contaminating reagents. The EchoLUTION Viral RNA/DNA Swab Kit is compatible with dry swabs and swabs in transport media. For product specifications, see table 1.

Table 1: BioEcho technology and product specifications

EchoLUTION Viral RNA/DNA Swab Kit	
Extraction technology	EchoLUTION
Sample material	< 50 µL of resuspended dry swabs < 90 µL swabs in transport media
Target nucleic acid	Viral RNA and viral DNA
Elution volume	90 µL

The EchoLUTION Viral RNA/DNA Swab Kit is compatible with ten different transport media. For compatibility information, see table 2.

Hamilton Microlab STAR Liquid Handling System

The EchoLUTION Viral RNA/DNA Swab Kit automated protocol was developed for the Hamilton Microlab STAR liquid handling system (figure 1). This workflow allows the extraction of viral RNA from 384 patient samples, including PCR setup, in less than one and a half hours.

Table 2: Routine swab transport media compatible with the EchoLUTION Viral RNA/DNA Swab Kit

Manufacturer	**Name**	**Type**
BioEcho Life Sciences GmbH	EchoSAFE Viral Transport Medium	chaotropic
CoWin Biosciences	Viral Sample Preservation Solution	chaotropic
Prestige Diagnostics	Single-Use Specimen Container	chaotropic
Procomcure Biotech	Phoenix Protect DNA/RNA Conversation Solution	chaotropic
Roche	cobas® PCR Media	chaotropic
Copan	eSwab®	non-chaotropic
Copan	UTM®	non-chaotropic
Heinz Herenz Germany	LMS-SWAB	non-chaotropic
Hologic	Specimen Lysis Tube	non-chaotropic
PBS or TRIS Buffer	in-house production	Resuspension of dry swabs

Figure 1: Hamilton Microlab STAR Liquid Handling System automated for viral RNA and DNA extraction

Furthermore, this protocol is compatible with the other members of the Hamilton Microlab STAR line, including the Microlab STARlet. Table 3 describes the product specifications of the Hamilton Microlab STAR.

Table 3: Hamilton technology and product specifications

Hamilton Microlab STAR Liquid Handling System	
Extraction technology	Automated liquid handling platform with air displacement pipetting technology
Capacity*	4 × 96 purification samples 4 × 96 PCR plate 1 × 384 PCR plate
Pipetting volume	1 - 1,000 µL
Equipment/Features	MultiProbe Head (MPH), 8 pipetting channels, CO-RE (Compressed O-Ring Expansion) technology, liquid level detection, autoload barcode scanner, and iSWAP plate handler

*Capacity might change according to the Hamilton equipment selected. The Hamilton Microlab STARlet (a more compact member of the Microlab STAR line) has a capacity of 2 × 96 samples per run.

Materials and methods

The CE-IVD EchoLUTION Viral RNA/DNA Swab Kit developed for viral extraction from patient samples is compatible with dry swabs and swabs in transport medium. For swabs in a transport medium, such as Copan UTM or eSwab, or EchoSAFE Viral Transport Medium, among others, we prepared the lysis plate with between 50 µL and 70 µL LyseNtact lysis buffer per well. We then added the sample in swab transport media in a ratio of 1:1 with the LyseNtact buffer and mixed by pipetting. The viral particles are lysed instantly, without further incubation steps.

Though BioEcho does not provide internal controls, some PCR manufacturers specify the addition of internal controls in their protocols, so we added 20 µL of the internal control to each well when testing those products, taking into account that controls added before the purification step need to be at least 500 nucleotides long.

We then transferred 90 µL of the lysate into the purification plate containing the matrix, which we had

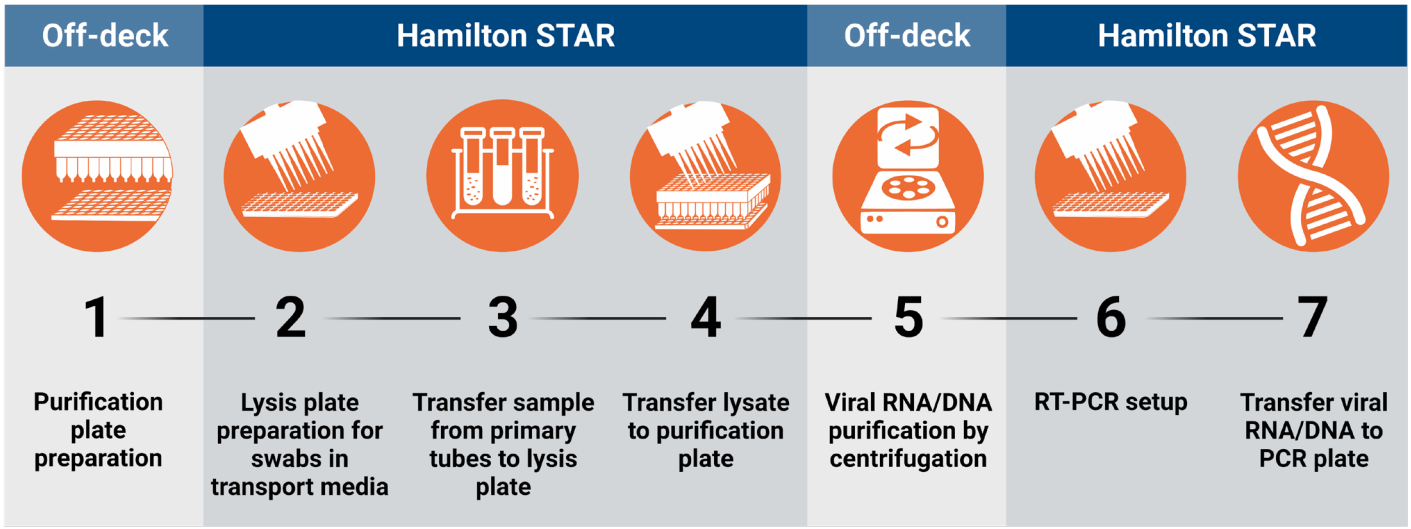

Figure 2: Illustration of the EchoLUTION Viral RNA/DNA Swab Kit automated workflow on the Hamilton Microlab STAR Liquid Handling System

conditioned previously by centrifugation, and purified the viral RNA and DNA using single-step centrifugation. In parallel to the centrifugation step, we ran the PCR setup. Finally, we transferred the eluate containing the viral nucleic acids into the PCR plate for downstream analysis.

For dry swabs, we resuspended the swab in a volume of 300 µL to 700 µL of EchoSAFE Viral Transport Medium as recommended. Having removed and discarded the swabs, we loaded the samples into the liquid handler, and the process after that was the identical to that for swabs in transport medium. All centrifugation steps took place outside the Hamilton Microlab STAR liquid handler.

When combined with the automated system from Hamilton, the EchoLUTION technology allowed us to process 4 × 96 samples, including PCR setup, in one hour 30 minutes for dry swabs and swabs in transport medium, after swab removal. The tailored protocol allowed us to set up the PCR in 96 or 384-well plates, affording increased precision and sample throughput. For a detailed explanation of the automated protocols, see figure 2.

Results

We validated the automated workflow of the EchoLUTION kit for both dry swabs and swabs in transport media. We then evaluated the sensitivity of the product and method with lyophilized SARS-CoV-2 samples obtained from the interlaboratory comparison trial organized by the INSTAND e.V. (Düsseldorf, Germany). Furthermore, we did not find cross-contamination. Sensitivity and cross-contamination results are represented in figure 3 and figure 4, respectively.

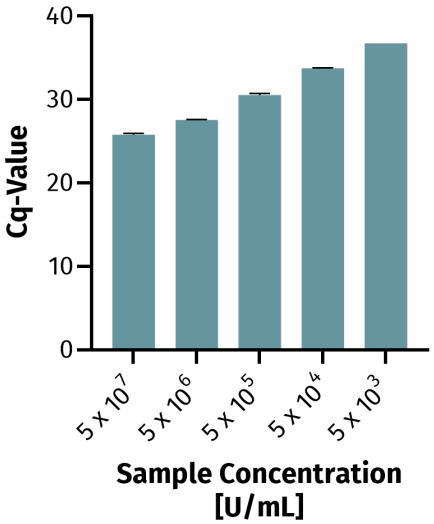

Figure 3: High sensitivity detection of SARS-CoV-2 RNA with the automated EchoLUTION viral workflow. SARS-CoV-2 lyophilized viral particles were extracted with the EchoLUTION Viral RNA/DNA Swab Kit automated protocol. Samples were analyzed by RT-qPCR using the cobas SARS-CoV-2 test from Roche. Bars represent mean and ±SE (n = 2 for each dilution).

	1	2	3	4	5	6
A	20.4	-	27.5	-	19.4	-
B	-	21.7	-	19.4	-	19.6
C	22.3	-	25.6	-	16.9	-
D	-	28.5	-	20.5	-	23.5
E	17.7	-	23.9	-	18.6	-
F	-	22.4	-	21.2	-	22.4
G	27.3	-	17.6	-	20.9	-
H	-	26.3	-	21.4	-	28.5

Figure 4: The EchoLUTION viral automated protocol did not exhibit cross-contamination. Confirmed positive SARS-CoV-2 samples (n = 24) and negative samples (n = 24) from a total of 48 different patients were extracted with the EchoLUTION Viral RNA/DNA Swab Kit. Samples were arranged following a checkerboard pattern and analyzed by RT-qPCR using the cobas SARS-CoV-2 test from Roche. All positive samples were successfully amplified. Negative samples present an undetermined CT value (-).

Speed up the viral extraction process of your laboratory

The EchoLUTION Viral RNA/DNA Swab Kit provides a rapid and reliable solution for diagnostic laboratories, allowing them to conduct large-scale tests for COVID-19 diagnosis in a shorter time. The automated workflow based on instant virus lysis without incubation, followed by one-minute single-step centrifugation for purification, is the fastest solution available on the market for viral nucleic acid extraction (figure 5).

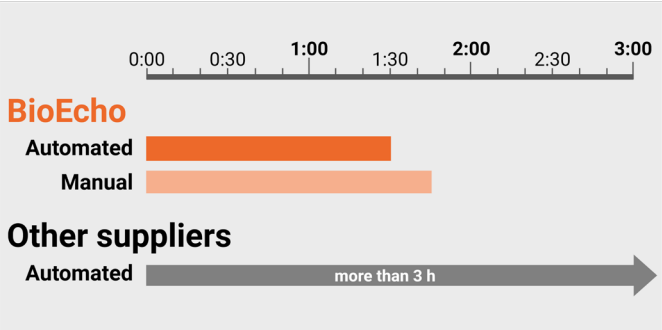

Figure 5: EchoLUTION Viral RNA/DNA Swab Kit automated workflow is faster than manual extraction and other suppliers. After swab removal, the semi-automated workflow from BioEcho is more than two times faster than other automated solutions on the market. The timelines illustrate the time required for processing 4 × 96 samples, including PCR setup, with the BioEcho methods (automated and manual) and other suppliers. For both BioEcho methods, the timelines include the centrifugation steps for purification plate preparation and viral RNA/DNA purification.

This method will allow you to:

- Obtain reliable, reproducible, sensitive, and traceable results without cross-contamination
- Extract 348 samples, including PCR setup, in less than one and a half hours
- Simplify your workflow while increasing your throughput

Ordering Information

Product	Reactions	Product No.
EchoLUTION Viral RNA/DNA Swab Kit Plus: For single-step 48- well purification of viral RNA & DNA from nasopharyngeal swabs. Starting material: dry, non-resuspended or swabs in stabilization medium (half-filled 96-well purification plates).	2 x 48 8 x 48 16 x 48	012-051-002 012-051-008 012-051-016
EchoLUTION Viral RNA/DNA Swab Kit Plus: For single-step 96-well purification of viral RNA & DNA from nasopharyngeal swabs. Starting material: dry, non-resuspended or swabs in stabilization medium.	2 x 96 8 x 96 16 x 96	012-102-002 012-102-008 012-102-016
EchoSAFE Transport Medium: Virus inactivating medium, compatible with EchoLUTION viral RNA extraction kits; for safe transport of viral swabs.	500 mL 20 L	030-004-005 030-004-020
Hamilton Microlab® STAR™ Liquid Handling System: Automated liquid handling platform with MultiProbe Head (MPH), 8 pipetting channels, CO-RE gripper, autoload barcode scanner, iSWAP plate handler. Optimized for nucleic acid purification and PCR setup.		Hamilton

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