APPLICATION NOTE

CE IVD

Validation of the EchoLUTION™ Viral RNA/DNA Swab Kit Plus for Fast and Reliable SARS-CoV-2 Extraction

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Although innovative biotechnologies are continuously developed, the method of nucleic acid extraction remained unchanged for several decades. The classical bind-wash-elute approach previously seemed sufficient, but the COVID-19 pandemic uncovered the need for a new and faster technique for multi-parallel processing. BioEcho developed a completely new technology, which simplifies and speeds up the process of viral RNA extraction: the EchoLUTION technology. This technology is based on a one-step isolation: all impurities are retained in the matrix, while the RNA and DNA pass through it without further interactions and without the need of organic solvents. In this study, we assay the performance of the CE-IVDR-certified EchoLUTION Viral RNA/DNA Swab Kit Plus for SARS-CoV-2 diagnostic.

Introduction

The COVID-19 pandemic has hit the whole world with an unforeseeable impact, and it might not be the last viral pandemic we will face. While it is extremely important to focus on vaccinations and treatments against SARS-CoV-2, we also need to prevent the virus from spreading. SARS-CoV-2 RNA extraction is the first step in performing RT-qPCR–based methods for determining the presence of a current viral infection, monitoring infection and treatment courses, and supervise the emergence of new subtypes and mutations as part of epidemiologic evaluation and screening. However, conventional extraction methods are time-consuming due to tedious washing and elution steps. BioEcho Life Sciences GmbH has developed the EchoLUTION Viral RNA/DNA Swab Kit Plus, using a novel nucleic acid extraction technology, for research and in vitro diagnostic (IVD) applications in a professional laboratory setting. The innovative technology allows a much faster DNA and RNA purification compared to silica-based bind-wash-elute methods and ensures a high-quality and inhibitor-free throughput.

During the pandemic, up to 12 % and 50 % of SARS-CoV-2 PCR test have been performed in Germany and Austria, respectively, using the EchoLUTION Viral RNA/DNA Swab Kit Plus. The kit is available in a well-plate format for the isolation of 48 or 96 samples. Its principle is based on a

lysis step without incubation time followed by a single-step isolation. In contrast to conventional methods, nucleic acids are not bound to a membrane or magnetic beads but instead migrate freely and untouched through the purification matrix. Unwanted components of the viral lysate are removed from the sample and remain in the matrix. 96 samples can be extracted within 20 minutes (comprising the entire workflow, which includes pipetting), meaning it is ten times faster than other silica-based methods.

This application note describes the validation of the EchoLUTION Viral RNA/DNA Swab Kit Plus, highlighting the kit's convenient workflow and high clinical performance. Being the kit purpose the isolation of viral RNA and DNA (depending on the viral particle to be detected) from nasopharyngeal and stool specimens, here, we report a series of experiments that demonstrate the analytical efficacy of the kit and its performance in the context of nasopharyngeal specimens collected from SARS-CoV-2 positive patients and healthy controls.

Materials and Methods

Swabs and transport media

Standard sampling for SARS-CoV-2 testing uses nasopharyngeal swabs in tubes containing either inactivating and stabilizing (chaotropic) or only stabilizing media (non-chaotropic). The media we used to test compatibility were: viral transport medium (VTM, inhouse), Viral Sample Preservation Solution (CoWin Biosciences, U.S.), Single-Use Specimen Container (Prestige Diagnostics, U.K.), PhoenixProtect DNA/RNA Conservation Solution (Procomcure Biotech, Germany), Cobas® PCR Media (Roche®, Switzerland), eSwab® and UTM® (Copan, Italy), LMS-SWAB (Heinz Herenz, Germany), Specimen Lysis Tube (Hologic®, Germany), DNA/RNA Shield™ (Zymo Research, Germany), and inhouse-produced medium, as PBS or Tris.

Analytical performance

We evaluated analytical performance of the EchoLUTION Viral RNA/DNA Swab Kit Plus using heat-inactivated SARS-CoV-2 virus particles obtained from INSTAND e.V. (Düsseldorf, Germany). These SARS-CoV-2 virus particles are based on cell culture supernatants from Vero cells infected with SARS-CoV-2. We performed the analytical performance studies either with swabs in our viral transport medium (VTM) (inactivating and stabilizing) or in non-chaotropic eSwab (Copan) medium.

We extracted the RNA from inactivated virus particles using the EchoLUTION Viral RNA/DNA Swab Kit Plus according to the protocol. To generate a background that more closely mimics real patient samples, we spiked all test samples with saliva swab samples from healthy donors. For downstream analysis of the extracted SARS-CoV-2 RNA, we used the RIDA®GENE SARS-CoV-2 qPCR (R-Biopharm, Germany). We performed reverse transcriptase qPCR (RT-qPCR) on the Rotor-Gene 3000 Instrument (Corbett Life Science, now QIAGEN®, Germany) and the CFX Opus 96 Real-Time PCR Detection System (BioRad, Germany).

We assessed the dilutional linearity for the EchoLUTION Viral RNA/DNA Swab Kit Plus by undertaking a calibration plot with 11 different SARS-CoV-2 virus titers (1×10^3 to 1×10^5 copies/mL). We investigated each dilution level with VTM and eSwab (Copan) medium, respectively. We extracted the samples using the EchoLUTION Viral RNA/DNA Swab Kit Plus.

Limit of detection (LoD) and limit of quantification (LoQ)

According to the validation of analytical procedures from the European Medicine Agency (ICH Q2(R2)[1]), we calculated the LoD and the LoQ based on the standard deviation of the response and the slope. The detection limit is expressed as: $LoD = 3.3\ \sigma/S$, and the quantification limit as: $LoQ = 10\ \sigma/S$, where σ = the standard deviation of the response and S = the slope of the calibration curve. We used a serial dilution ranging from 1×10^3 to 1×10^5 copies/mL.

Intra- and inter-run precision

To determine RT-PCR intra- and inter-run precision, we spiked swab transport media with SARS-CoV-2 particles. We tested two different virus titers to mimic a high viral load (1×10^5) and a low viral load (2.5×10^3) with VTM. Overall, we analyzed 144 data points.

Clinical performance

Our collaborators provided a total of 370 patient samples data for clinical validation of the EchoLUTION Viral RNA/DNA Swab Kit Plus. We compared clinical performance of the EchoLUTION kit with other established methods (MagNA Pure 96® well from Roche, QIAamp® Viral RNA Mini Kit from QIAGEN, EasyMAG® from BioMérieux, Cobas z 480 from Roche, and GeneXpert® from Cepheid®), for the extraction of SARS-CoV-2 RNA. Besides, we analyzed different target genes (E gene, RdRP gene, N gene, and S gene) and swabs in transport media as well as dry swabs.

Checkerboard contamination test

To test cross-contamination between wells we performed a checkerboard contamination assay. For this, they loaded SARS-CoV-2 samples onto the first three columns of a 96-well plate in a checkerboard layout. Every other well served as a negative control without SARS-CoV-2 RNA. We analyzed all samples via qPCR to evaluate the occurrence of cross-contamination.

Results

Compatibility of transport media with the EchoLUTION Viral RNA/DNA Swab Kit Plus

We tested the compatibility of the transport media listed in Table 1 with the EchoLUTION Viral RNA/DNA Swab Kit Plus. None of the media listed have a lytic effect on pathogens. Except for the transport medium from Zymo, all tested media listed are compatible with the EchoLUTION Viral RNA/DNA Swab Kit Plus. For in-house produced transport media, care must be taken to ensure that they are sufficiently buffered in the pH range 7.2 ± 0.2. If this is not the case, downstream applications may be negatively affected.

Assessment of linearity

As for subsequent experiments, samples were spiked with five different SARS-CoV-2 virus titers in a dilution series (1 x 10^3 to 1 x 10^5 copies/mL) to analyze the linear range using the non-chaotropic eSwab (Copan) medium (Figure 2). Dilutional linearity determines whether the method can still provide reliable quantification after sample dilution. Samples displaying little to no deviation in concentration after factoring dilutions are known to display linearity. We determine that our sample dilutions displayed a linear relationship between expected and observed SARS-CoV-2 concentration showing a correlation coefficient of 0.9991 (Figure 1). These results indicate that the EchoLUTION RNA/DNA Viral Swab Kit Plus in combination with the RIDA®GENE SARS-CoV-2 (R-Biopharm) displays a high degree of accuracy and shows flexibility of the assay at varying concentrations.

Table 1. Compatibility of routine swab transport media with the EchoLUTION Viral RNA/DNA Swab Kit Plus.

Manufacturer	Name	Type	Compatibility with BioEcho
BioEcho Life Sciences GmbH	Viral Transport Medium (VTM)	Chaotropic	Yes
CoWin Biosciences	Viral Sample Preservation Solution	Chaotropic	Yes
Prestige Diagnostics	Single-Use Specimen Container	Chaotropic	Yes
Procomcure Biotech	PhoenixProtect DNA/RNA Conservation Solution	Chaotropic	Yes
Roche	Cobas PCR Media	Chaotropic	Yes
Copan	eSwab	Non-chaotropic	Yes
Copan	UTM	Non-chaotropic	Yes
Heinz Herenz Germany	LMS-SWAB	Non-chaotropic	Yes
Hologic	Specimen Lysis Tube	Non-chaotropic	Yes
Zymo Research	DNA/RNA Shield™	Non-chaotropic	No
PBS or TRIS Buffer	In-house production	Resuspension of dry swabs	Yes

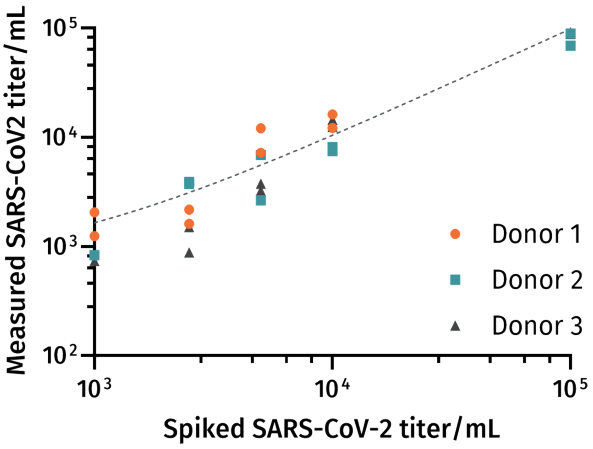

Figure 1. Confirmation of linearity using SARS-CoV-2 virus particles diluted in eSwab medium containing saliva swab material. *Linear correlation is shown for the mean value of three biological replicates (three healthy donors provide the saliva as matrix) determined with two technical replicates each (grey dotted line).*

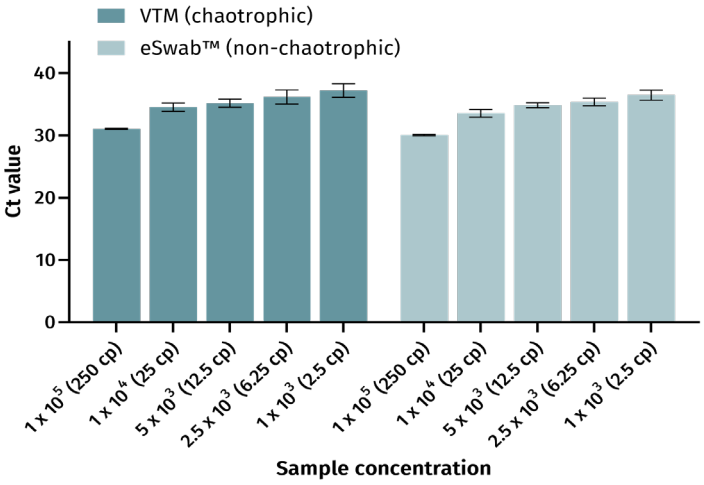

Figure 2. The EchoLUTION Viral RNA/DNA Swab Kit Plus is compatible with chaotropic and non-chaotropic transport media. *Bar chart analysis shows representative results for VTM (dark) and eSwab (light) medium. Standard deviation is shown for each SARS-CoV-2 dilution. Copies (cp) shown in brackets indicate the concentration per RT-qPCR reaction using the RIDA®GENE SARS-CoV-2 (R-Biopharm) assay. 5 µL of each sample was used for RT-qPCR.*

Next, we diluted SARS-CoV-2 virus particles ranging from 1 x 10^3 to 1 x 10^5 copies/mL with either eSwab medium or viral transport medium (VTM), each containing pooled saliva swab material from six healthy individuals. We prepared and purified the samples according to the protocol using the EchoLUTION Viral RNA/DNA Swab Kit Plus. We tested each dilution with eight technical replicates resulting in 80 overall data points (Figure 2). The Ct values of decreasing SARS-CoV-2 sample concentrations increased within the expected range and were comparable between samples prepared with the VTM, which is chaotropic (inactivating and stabilizing), or the eSwab medium (Copan), which is non-chaotropic. A SARS-CoV-2 titer of 2500 copies/mL (12.5 cp/assay) was not measurable in two and one sample diluted with VTM and eSwab, respectively, while a SARS-CoV-2 titer of 1000 copies/mL (5 cp/assay) was not measurable in three and four samples diluted with VTM and eSwab, respectively. The data show that both swab transport media are compatible for SARS-CoV-2 RT-qPCR detection following the EchoLUTION extraction process.

Evaluation of RT-qPCR sensitivity

We next assessed RT-PCR sensitivity determining the limit of detection (LoD) and the limit of quantification (LoQ) for the EchoLUTION Viral RNA/DNA Kit Plus in combination with the RIDA®GENE SARS-CoV-2 (R-Biopharm). We used the standard deviation of intercept (σ) and the slope (s) of the standard curve within the linear range (1 x 10^3 to 1 x 10^5 copies/mL), to calculate the LoD and the LoQ (Table 2). The results show that the workflow allows highly sensitive detection of SARS-CoV-2 infections, with a limit of detection of approximately 2,000 viral copies per mL of transport medium.

Table 2. Limit of detection (LoD) and limit of quantification (LoQ) of the EchoLUTION Viral RNA/DNA Swab Kit Plus in combination with the RIDA®GENE SARS-CoV-2 RT-qPCR assays. *Cp = virus copies.*

MPCR Assay	Results
LoD (cp/mL)	1982
LoD (cp/µL eluate)	5
LoQ (cp/mL)	6007
LoQ (cp/µL eluate)	15

Table 3. Intra-run precision. *The table summarize the data from 24 spiked samples (1 x 10^5 cp/mL and 2.5 x 10^3 cp/mL, respectively) in a single run. SD: standard deviation; CV: coefficient of variation.*

	Single run		
Concentration (copies/mL)	**Mean (Ct)**	**SD (Ct)**	**CV (%)**
1×10^5	29.77	0.27	0.92
2.5×10^3	35.64	2.32	6.50

Table 4. Inter-run precision. *The table summarize the data from three independent runs. We used 24 samples for 1 x 10^5 cp/mL and 24 samples for 2.5 x 10^3 cp/mL for each run. SD: standard deviation; CV: coefficient of variation.*

	Run A-C		
Concentration (copies/mL)	**Mean (Ct)**	**SD (Ct)**	**CV (%)**
1×10^5	29.83	0.27	0.89
2.5×10^3	35.75	2.44	6.82

Intra- and inter-run precision

In order to express the precision, or repeatability of the SARS-Cov-2 detection with the EchoLUTION Viral RNA/DNA Kit Plus, we determine the CV (coefficient of variation) from intra- and inter-run assays. We determined the intra-run precision in three different runs and for two different virus concentrations each (Table 3). To determine the inter-run precision, data were produced in three independent runs (Table 4). In both cases, the CV is < 1 % for the high concentration samples. For low concentration, the intra- and inter-runs CV were < 7 % for the target concentrations tested.

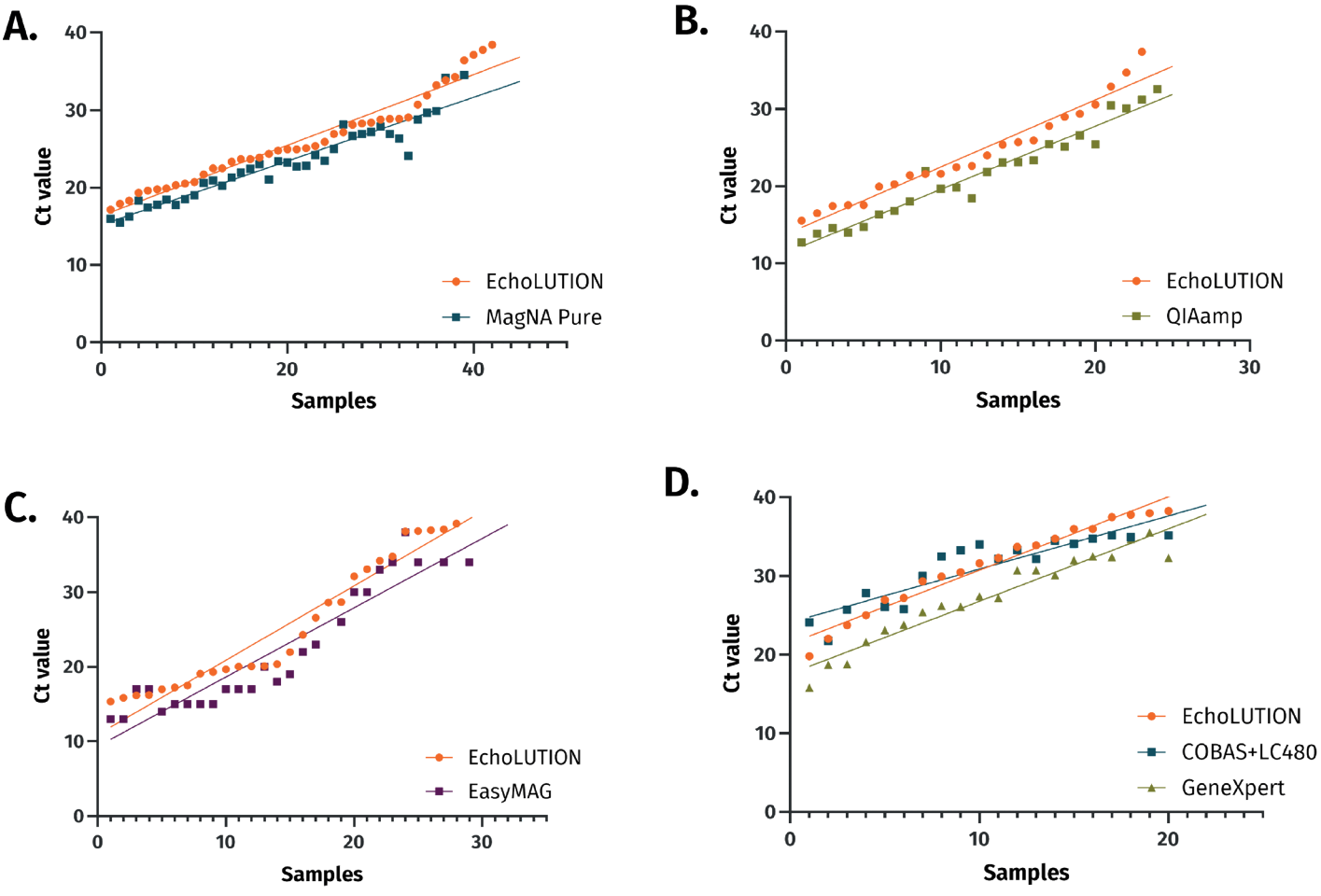

Figure 3. Clinical performance comparison between EchoLUTION Viral RNA/DNA Swab Kit Plus and other suppliers' kits for the extraction of SARS-CoV-2 RNA. ***A.*** *62 patients' samples were analyzed for the detection of the RdRP gene with EchoLUTION (orange) and MagNA Pure (blue) extraction kits.* ***B.*** *24 patients' samples were analyzed to target the N gene with EchoLUTION (orange) and QIAamp (green) extraction kits.* ***C.*** *28 patients' samples were analyzed for the detection of the E gene with EchoLUTION (orange) and EasyMAG (purple) extraction kits, respectively.* ***D.*** *EchoLUTION kit results were compared with Cobas (blue) and GenXpert (green) kits to detect E gene from SARS-CoV-2 in 20 patients' samples.*

SARS-CoV-2 extracted from clinical samples

To compare clinical performance of the EchoLUTION Viral RNA/DNA Swab Kit Plus with established methods for the extraction of SARS-CoV-2 RNA we compared the RT-PCR data with other vendor kits (MagNA Pure, Roche and QIAamp RNA Viral kit, QIAGEN). Clinical data shown in Figure 3 reveal a robust and reliable extraction with EchoLUTION Viral RNA/DNA Swab Kit Plus, which is similar to compared conventional methods. To mention that the Ct obtained with EchoLUTION Viral RNA/DNA Swab Kit Plus (data points in orange) are 2 – 3 cycles later compared to state-of-the-art methods (data points in different colors). However, the shift in Ct values is consistent and can easily be taken into consideration for interpretation of the results.

Table 5. Checkerboard contamination test. *Table represent the position of each positive sample in the plate and the Ct value from each one.*

	1 (Ct)	2 (Ct)	3 (Ct)
1	-	19.72	-
2	19.68	-	20.07
3	-	19.82	-
4	19.99	-	19.67
5	-	20.1	-
6	19.84	-	19.08
7	-	19.17	-
8	19.76	-	19.50
9	-	19.71	-
10	19.56	-	19.72
11	-	20.00	-
12	19.73	-	19.66

Checkerboard contamination test

CERTE Medical Diagnostics and Advise (Netherlands) validated the EchoLUTION Viral RNA/DNA Kit Plus for internal purposes and kindly provided their data from a checkerboard contamination test. Thirty-six EchoLUTION extractions were performed, alternating 18 positive (~Ct = 19) and 18 negative samples (VTM without patient material) in a “checkerboard” pattern. Results are depicted in Table 5, where no cross-contamination could be observed.

Discussion

One of the first steps in processing samples for the detection of viral infections is the RNA (or DNA) extraction step. The SARS-CoV-2 pandemic has demonstrated the need for fast and reliable validated kits in the market. The EchoLUTION Viral RNA/DNA Swab Kit Plus presents an innovative technique for accelerating the extraction step. The workflow for the EchoLUTION RNA/DNA Viral Swab Kit Plus is straightforward as shown in Figure 1 and the fastest method available. The procedure involves a lysis step without incubation, transfer of the lysate, and a single-step purification. The innovative EchoLUTION technology reduces the extraction time with consistent sensitivity compared to state-of-the-art methods. The speed is a benefit enabling clinicians to consider timely critical interventions or to rule out an infection. Also, the kit enables for high sample throughputs while having low equipment and capital investment. Together with a generic protocol for common swab media and dry swabs, this forms an important clinical benefit.

In summary, we demonstrated that the EchoLUTION RNA/DNA Viral Swab Kit Plus is compatible with standard transport media as well as with dry swabs and provide validation as well as performance data of the kit in analytical and clinical setups. SARS-CoV-2 RNA can successfully be detected down to 5 copy/µL eluate with the EchoLUTION Viral RNA/DNA Swab Kit Plus in combination with RIDA®GENE SARS-CoV-2 RT-qPCR. The slightly higher Ct can be easily explained because the EchoLUTION Viral RNA/DNA Swab Kit Plus do not concentrate the samples as much as other comparable kits on the market. However, these values do not have an influence on results reliability, and they are not of clinical importance. The inter- and intra-run precision is within an appropriate validation range and no cross-contamination could be observed.

The validation data together with the fact that the extraction is roughly ten times faster compared to conventional silica-based extraction methods

demonstrate that the CE-IVD-certified EchoLUTION Viral RNA/DNA Swab Kit Plus represents an attractive solution for SARS-CoV-2 and other pathogenic viruses RNA/DNA extractions in diagnostic settings.

NOTE: BioEcho has introduced a new kit, the EchoLUTION Viral RNA/DNA Kit, which contains a modified lysis buffer (LyseNtact Buffer New Formula).

In the application note ***Evaluation of the new EchoLUTION Viral RNA/DNA Kit for nucleic acid extraction of respiratory and enteropathogenic viruses***[2], we demonstrate that the performance of the new kit is the same as that of the EchoLUTION Viral RNA/DNA Swab Kit Plus. Accordingly, all data shown in this application note are still applicable.

References

[1] EMA (2022) ICH Q2(R2) Validation of Analytical Procedures - Scientific Guideline. European Medicines Agency. Available at: https://www.ema.europa.eu/en/ich-q2-r1-validation-analytical-procedures-text-methodology

[2] Maximilian Weiter, Rico Schulze, Vera Kloten, Maika Schiwy, Christoph Schönfels, and Laura Torres Benito. *Evaluation of the new EchoLUTION Viral RNA/DNA Kit for nucleic acid extraction of respiratory and enteropathogenic viruses*. 2023. Published as an application note by BioEcho Life Sciences GmbH.

Ordering Information

Product	Reactions	Product No.
EchoLUTION Viral RNA/DNA Kit	2 × 48 8 × 48 16 × 48	012-051-002-Dx 012-051-008-Dx 012-051-016-Dx
	2 × 96 8 × 96 16 × 96	012-102-002-Dx 012-102-008-Dx 012-102-016-Dx

*The EchoLUTION Viral RNA/DNA Swab Kit Plus has been replaced with the new EchoLUTION Viral RNA/DNA Kit. The difference between the kits is the modified lysis buffer (LyseNtact Buffer New Formula), all other components of the new kit stayed the same.

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