June 2022

EZ1® DSP Virus Kit
Instructions for Use (Performance Characteristics)

Version 5

IVD

For In Vitro Diagnostic Use
For use with EZ1 DSP Virus Kit (48)

CE

REF 62724

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R1 Performance Characteristics available electronically and can be found under the resource tab of the product page on **www.qiagen.com**.

Sample to Insight

General Introduction

The EZ1 DSP Virus Kit is intended for purification of viral nucleic acids and bacterial DNA from plasma, serum, CSF, stool, and nasopharyngeal swabs collected in Universal Transport Medium™ (UTM®). Magnetic particle technology provides high-quality nucleic acids (NA) that are suitable for direct use in downstream applications, such as PCR and qPCR amplification. The EZ1 and EZ2® Connect MDx instruments perform all steps of the sample preparation procedure for up to 6 samples (using the EZ1 Advanced or the BioRobot® EZ1 DSP, both discontinued), for up to 14 samples (using the EZ1 Advanced XL) or for up to 24 samples (using the EZ2 Connect MDx) in a single run.

The sample input volume can be chosen from 100, 200 or 400 µl, and the NA elution volume can be chosen from 60, 90, 120, or 150 µl.

The EZ1 DSP Virus Kit system performance has been established in performance evaluation studies using plasma, serum, CSF, stool, and nasopharyngeal swabs collected in UTM for isolation of viral NA and bacterial DNA. However, kit performance is not guaranteed for each virus or bacteria species and must be validated by the user. It is the user's responsibility to validate system performance for any procedures used in their laboratory that are not covered by the QIAGEN® performance evaluation studies.

Performance Characteristics of EZ1 Instruments

Note: Performance Characteristics highly depend on various factors and relate to the specific downstream application. Performance has been established for the EZ1 DSP Virus Kit in conjunction with exemplary downstream applications. However, methods for isolating nucleic acids from biological specimen are used as a front-end for multiple downstream applications. Thus, performance parameters such as the influence of exogenous interfering substances, cross contamination, or run precision need to be established for any such workflow as part of the downstream application development. Therefore, it is the responsibility of the user to validate the whole workflow to establish appropriate performance parameters.

Basic performance and compatibility to different downstream applications

Various different primary tubes and anticoagulants can be used to collect blood samples for the EZ1 DSP Virus procedure. Basic performance for the EZ1 DSP Virus Kit was evaluated using 6 single donors for viral NA extraction from 4 different blood collection tubes. Table 1 provides an overview of the sample collection tubes that have been used for evaluation of the system. After plasma or serum preparation, the samples were spiked with a dedicated virus titer of hepatitis C (HCV) or hepatitis B (HBV). Using suitable qPCR systems, the virus titer was determined for each sample. The average virus titer using different primary tubes are shown in Figure 1.

Table 1. Blood collection tubes tested with the EZ1 DSP Virus system

Primary tube	Manufacturer	Cat. no.*	Preservative/anticoagulant
BD™ Vacutainer® PTT	BD	362788	K2EDTA – gel – plasma
BD Vacutainer K2E	BD	367525	K2EDTA – plasma
S-Monovette® 9NC	Sarstedt®	02.1067.001	Sodium citrate – plasma
S-Monovette Serum Gel Z	Sarstedt	02.1388.001	Gel – serum

* Catalog numbers are subject to change; please check with the manufacturer or supplier.

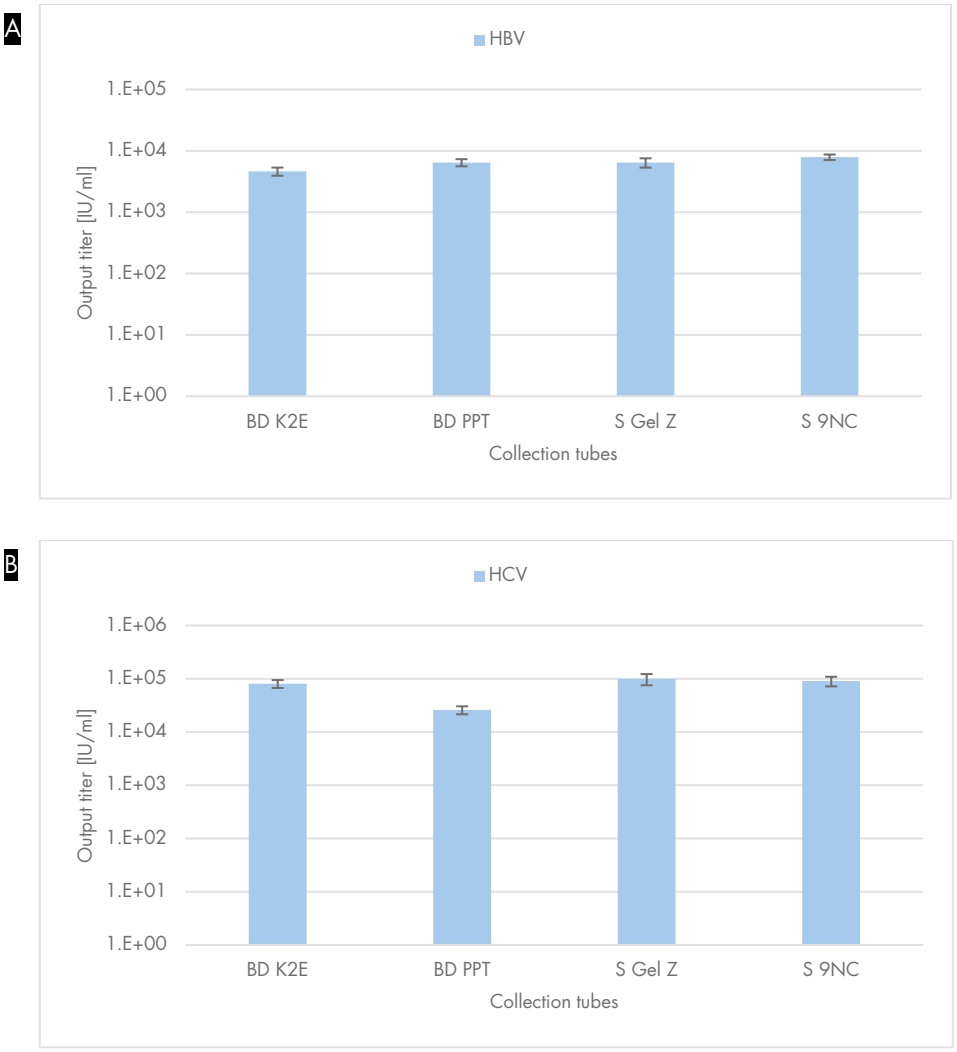

Figure 1. Basic performance using different collection tubes and anticoagulants. Blood samples were collected from 6 healthy donors in different types of tubes to prepare either plasma or serum with replicates of 10 per donor tube. The tubes used are listed in Table 1 (BD: Becton Dickinson, S: S-Monovette). **A**: Viral DNA was purified from 200 µl samples, with elution in 90 µl. **B**: Viral RNA was purified from 200 µl samples with elution in 90 µl. NA yields from each donor and tube were determined by qPCR analysis. The bars show the mean virus titer outputs with standard deviation.

The linear range for the EZ1 DSP Virus Kit was evaluated using Adenovirus 5 as a DNA virus spiked into stool samples. The tests were performed with serial 10-fold dilutions of cell culture supernatant in Adenovirus negative stool. Dilution series with 5 different virus dilutions were tested with 10 replicates each. Viral nucleic acids were extracted from 200 µl samples (1:10 resuspended in Buffer ASL*) and eluted in 120 µl. The linear range of the EZ1 DSP Virus procedure has been determined in combination with a suitable qPCR assay in comparison to a spin-column based DNA extraction method (Figure 2).

* QIAGEN GmbH, cat. no. 190822

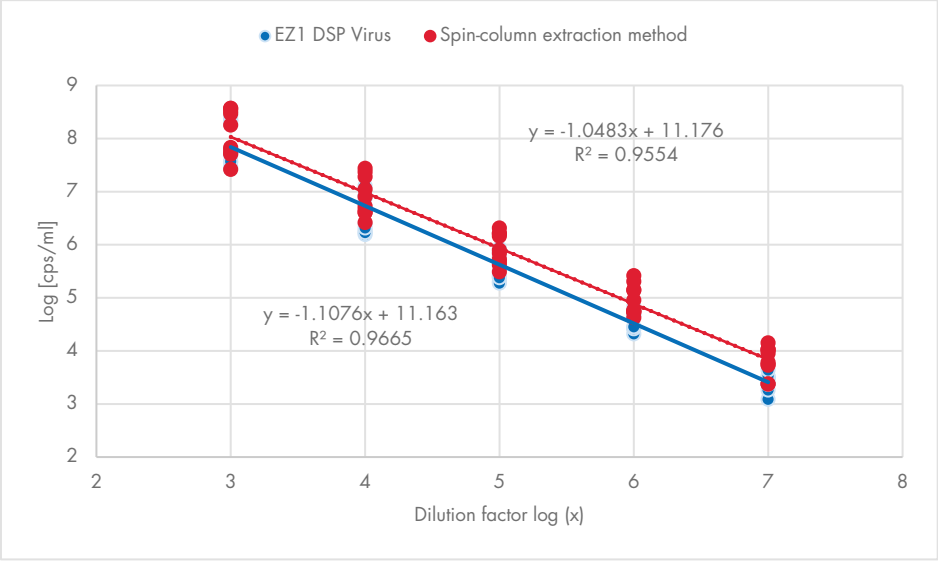

Figure 2. Linear range of virus titer using the EZ1 DSP Virus protocol. Shown are the results from a suitable Adenovirus PCR assay in combination with eluates from the extraction of Adenovirus 5 from stool samples, either using the EZ1 DSP Virus Kit or a spin-column based DNA extraction method.

Additional linear range data were generated by spiking cytomegalovirus (CMV) as a DNA virus into EDTA plasma samples prepared from 1 donor. Dilution series with 7 different virus dilutions were tested with 9 replicates each. Viral nucleic acids were extracted from 400 µl samples and eluted in 60 µl on the EZ1 Advanced XL. The linear range has been determined in combination with a suitable CMV PCR assay.

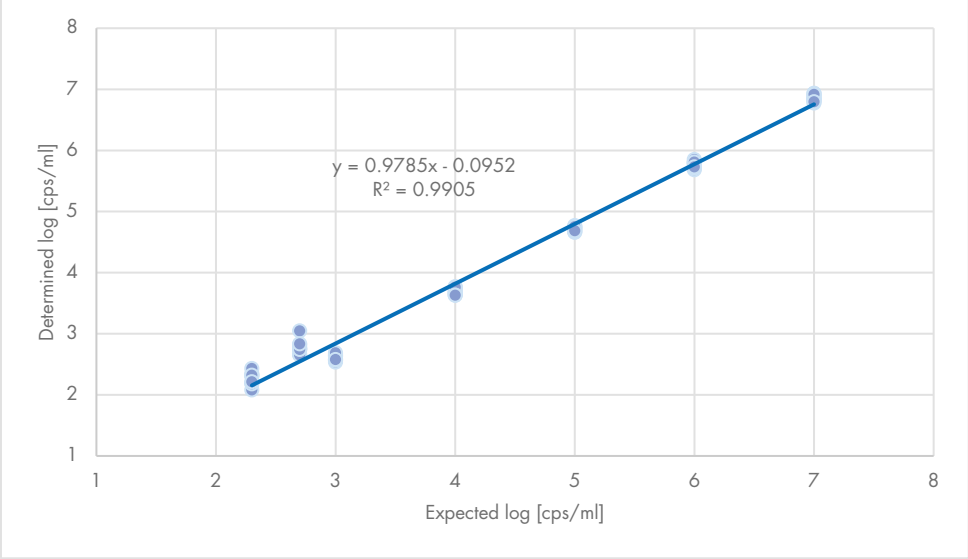

Figure 3. Linear range of virus titer using the EZ1 DSP Virus protocol. Shown are the results from a suitable CMV PCR assay in combination with eluates from the extraction of CMV from EDTA plasma samples.

NA eluates purified from different sample materials using the EZ1 DSP Virus system were analyzed and showed compatibility with different quantitative real-time PCR (qPCR) assays.

Freezing–thawing of samples

It is not recommended to refreeze thawed samples or store samples for over 6 hours at 2–8°C, as this leads to significantly reduced yields and quality of viral nucleic acids or bacterial DNA.

Precision

Standard deviations and CVs were determined for HIV-1 and CMV dilutions in the linear range of the appropriate downstream assays. NA was extracted from 400 µl plasma sample spiked with the respective virus material and eluted in 120 µl. In total, 7 purification runs per virus dilution were performed with one operator, on 3 instruments and on 3 different days. Eluates were analyzed using an HIV-suitable RT-PCR assay and a CMV PCR assay. The intra-run precision data are shown as standard deviation in Figure 4.

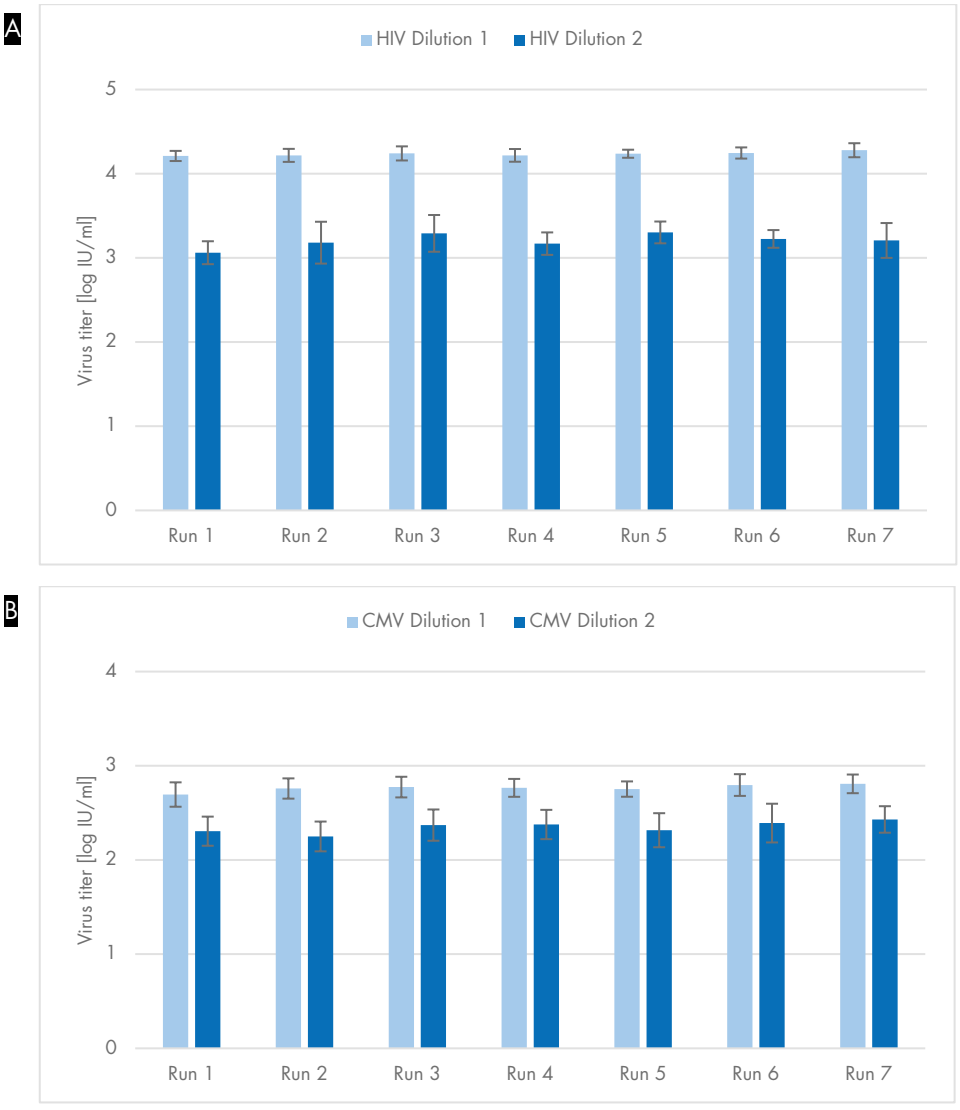

Figure 4. Intra-run precision using the EZ1 DSP Virus system. Plasma was collected, pooled, and prepared with the respective virus titer before use (**A**: HIV; **B**: CMV). NA was purified from 400 µl aliquots in 7 runs of 14 replicates each on the EZ1 Advanced XL using the EZ1 DSP Virus system. Mean virus titer and standard deviation are shown for each run.

CVs were determined for the extraction of NA from plasma samples. The precision data are shown in Table 2 and Table 3.

Table 2. Analysis of precision estimates – intra-run variability (HIV)

Precision (HIV)	CV (%) (Dilution 1)	CV (%) (Dilution 2)
Intra run (Run 1)	1.43	4.45
Intra run (Run 2)	1.83	7.82
Intra run (Run 3)	1.98	6.64
Intra run (Run 4)	1.79	4.21
Intra run (Run 5)	1.13	3.92
Intra run (Run 6)	1.56	3.27
Intra run (Run 7)	1.95	6.46

Table 3. Analysis of precision estimates – intra-run variability (CMV)

Precision (CMV)	CV (%) (Dilution 1)	CV (%) (Dilution 2)
Intra-run (Run 1)	4.81	6.71
Intra-run (Run 2)	3.90	7.03
Intra-run (Run 3)	3.95	7.01
Intra-run (Run 4)	3.44	6.54
Intra-run (Run 5)	2.96	7.81
Intra-run (Run 6)	4.13	8.60
Intra-run (Run 7)	3.53	5.79

In addition, inter-run variability was determined for both virus dilutions (Table 4).

Table 4. Analysis of precision estimates – inter-run variability (HIV, CMV)

Precision (CMV)	CV (%) (Dilution 1)	CV (%) (Dilution 2)
Inter-run (Run 1–7) HIV	1.72	5.81
Inter-run (Run 1–7) CMV	3.92	7.30

Standard deviations and coefficients of variations (CVs) for stool were determined for Adenovirus 5 using an Adenovirus compatible PCR assay. Adenovirus negative stool was spiked with Adenovirus 5 cell culture supernatant. Viral DNA was extracted from 200 µl samples (1:10 resuspension in Buffer ASL*) and eluted in 120 µl. In total, 7 purification runs were performed with one operator, on three EZ1 Advanced XL instruments, on 3 different days and 3 EZ1 DSP Virus Kit/Buffer ASL lot combinations. All samples were analyzed in the same PCR run. The intra-run precision data are shown as standard deviation in Figure 5.

* QIAGEN GmbH, cat. no. 19082

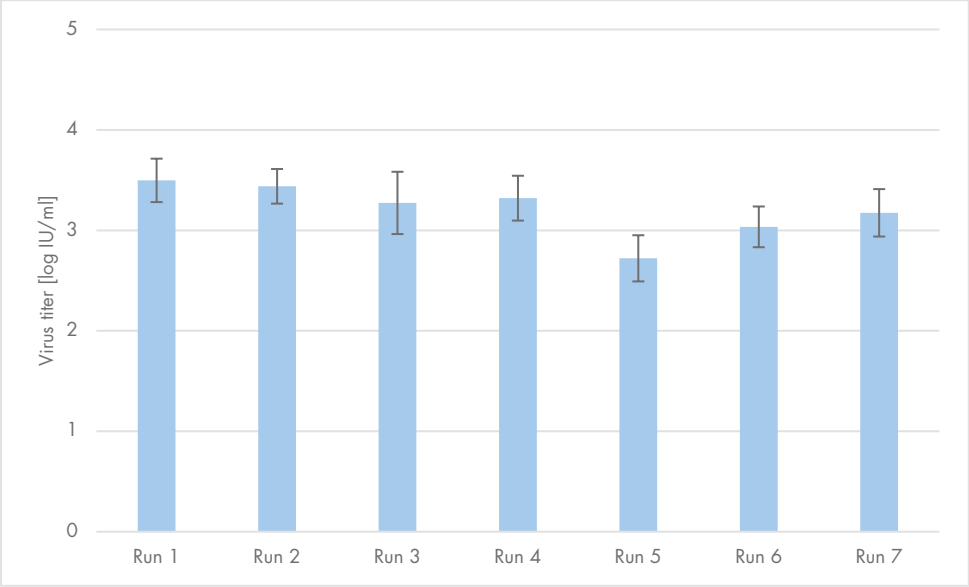

Figure 5. Intra-run precision using the EZ1 DSP Virus system. Stool samples were collected, pooled and prepared with the respective virus titer before use. NA was purified from 200 µl aliquots in 7 runs of 9/10 replicates each on the EZ1 Advanced XL. Mean virus titer and standard deviation are shown for each run.

CVs were determined for the extraction of NA from stool samples. The precision data are shown in Table 5.

Table 5. Analysis of precision estimates (Adenovirus 5) – intra-run variability

Precision (CMV)	CV (%)
Intra-run (Run 1)	6.56
Intra-run (Run 2)	5.31
Intra-run (Run 3)	10.05
Intra-run (Run 4)	7.13
Intra-run (Run 5)	8.96
Intra-run (Run 6)	7.09
Intra-run (Run 7)	7.84

In addition, inter-run variability was determined (Table 6).

Table 6. Analysis of precision estimates – inter-run variability

Precision	CV (%)
Inter-run (Run 1–7)	10.54

Standard deviations and CVs for transport media were determined for HSV-1 and *Chlamydia trachomatis* using a suitable HSV1 PCR assay and a suitable *C. trachomatis* PCR assay. Viral and bacterial DNA was extracted from 400 µl UTM and eluted in 60 µl. In total, 6 purification runs were performed from one operator, on 3 days with 3 EZ1 DSP Virus Kit lots. All samples were analyzed in the same PCR run. The intra-run precision data are shown as standard deviations in Figure 6.

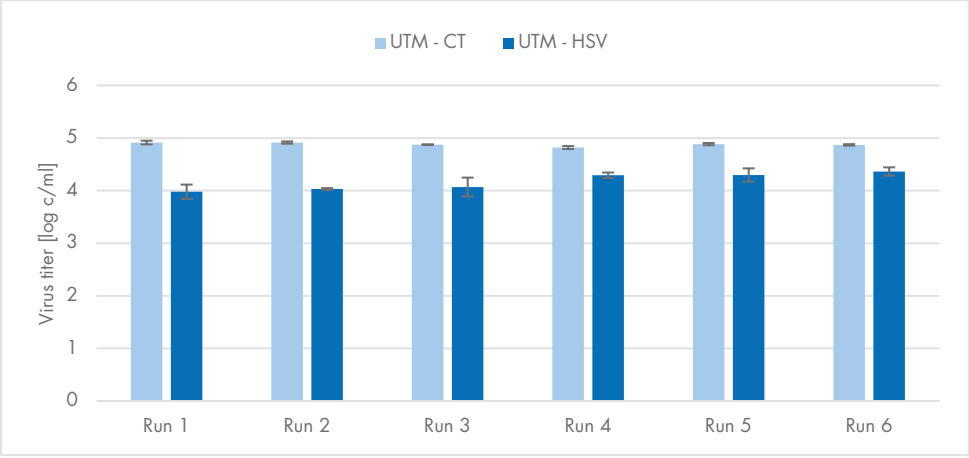

Figure 6. Intra-run precision using the EZ1 DSP Virus system. UTM was prepared with the respective virus titer before use. NA was purified from 400 µl aliquots in 6 runs of 2 replicates each on the EZ1 Advanced XL. Mean virus titer and standard deviation are shown for each run.

CVs were determined for the extraction of NA from UTM samples. The precision data are shown in Table 7.

Table 7. Analysis of precision estimates – intra-run variability (CT and HSV)

Precision (CMV)	CV (%) CT	CV (%) HSV
Intra-run (Run 1)	0.72	3.44
Intra-run (Run 2)	0.43	0.43
Intra-run (Run 3)	0.15	4.40
Intra-run (Run 4)	0.59	1.21
Intra-run (Run 5)	0.43	2.97
Intra-run (Run 6)	0.29	1.81

In addition, inter-run variability was determined (Table 8).

Table 8. Analysis of precision estimates – inter-run variability

Precision	CV (%) CT	CV (%) HSV
Inter-run (Run 1–6)	0.77	4.25

Sample input/eluate output

The EZ1 DSP Virus system on the EZ1 instrument family offers the possibility to combine different sample input volumes (100, 200, or 400 µl) with different eluate output volumes (60, 90, 120, or 150 µl). Overall performance of the extraction procedures used on the EZ1 instrument family has been verified by using different sample input and eluate output combinations possible.

The data of the different studies demonstrated that the yield of NA is highest with high sample input volumes in combination with high eluate output volumes. The concentration of NA is highest with high sample input volumes and low eluate output volumes. Depending on the complete workflow (sample preparation in combination with specific downstream application), there may be a most beneficial combination of sample input and elution volume that can help to optimize, for example, the final NA yield and concentration or to further minimize the potential influence of residual interfering substances. Different downstream applications even for the same sample material might require different sample input/eluate output combinations. Therefore, it is the user's responsibility to validate the whole workflow within their specific application to establish appropriate performance parameters.

Eluate stability

Eluate stability for the EZ1 DSP Virus Kit was evaluated using extracted viral RNA and DNA from human EDTA plasma samples. Eluates were stored at different temperatures and different time periods and were analyzed for stability using a validated in-house PCR assay.

The results demonstrated stability of nucleic acids for up to 24 hours when stored at 2–8°C, for up to 12 weeks when stored at –20°C, for up to 12 months when stored at –80°C.

Stability of nucleic acids might be different for the specific downstream application being used and needs to be self-validated by the user.

Interfering substances

The influence of exogenous interfering substances on the EZ1 DSP Virus system was analyzed by testing defined concentrations (3 times the acute peak concentration following drug therapeutic treatment, as recommended in the CLSI Guideline EP7-A2) of different substances (Table 9). These were spiked into EDTA-plasma samples either CMV-positive or CMV-negative and compared to interferent-negative plasma. NA eluates were analyzed using a suitable CMV PCR assay.

Note: Testing was done using exemplary downstream applications for an assessment of the quality of the extracted nucleic acids. However, different downstream applications may have different requirements with respect to purity (i.e., absence of potential interfering substances), so the identification and testing of relevant substances also needs to be established as part of the downstream application development for any workflow involving the EZ1 DSP Virus Kit.

Table 9. Test concentrations of potential interfering substances spiked into EDTA-plasma

Interfering substances	Final test concentration
Sulfamethoxazole	200 mg/l
Trimethoprim	5.2 mg/l
Claforan (Cefotaxime)	1 g/l
Tazobac (Piperacillin + Tazobactam)	Piperacillin: 1 g/l Tazobactam: 125 mg/l
Ticarcillin	1 g/l
Augmentin (Amoxicillin + Clavulanic acid)	Amoxicillin: 125 mg/l Clavulanic acid: 25 mg/l
Vancomycin	125 mg/l
Fluconazole	1 mg/l
Rapamycin	100 mg/l
Mycophenolate sodium	80 mg/l

All tested interfering substance concentrations showed no significant influence on the performance of the CMV PCR assay in combination with the EZ1 DSP Virus System with regards to specificity, sensitivity, and reliable quantification.

Additional testing of exogenous interfering substances using the EZ1 DSP Virus system was done by spiking defined concentrations of different substances (Table 10) into nasopharyngeal swabs collected in UTM. The sample material was spiked with influenza A and influenza B strains and NA eluates were analyzed using a suitable influenza A/B RT-PCR assay.

Table 10. Test concentrations of potential interfering substances being spiked into nasopharyngeal swabs collected in UTM

Interfering substances	Final test concentration
Human Blood	5% v/v
Zanamivir	3 mg/ml
Oseltamivir	15 mg/ml
NaCl with preservatives	10% v/v of sample
Phenylephrine	10% v/v of sample
Oxymetazoline	10% v/v of sample
Budesonide	40 µg/ml
Fluticasone propionate	2.5% v/v of sample
Luffa opperculata	4.5 mg/ml
Sulfur	4.5 mg/ml
Galphimia glauca	4.5 mg/ml
Histaminum hydrochloricum	4.5 mg/ml
Beclomethasone dipropionate	61.73 µg/ml
Flunisolide	25 µg/ml
Triamcinolone acetonide	27.5 µg/ml
Guaifenesin	1.33 mg/ml
Diphenhydramine hydrochloride	0.5 mg/ml
Dextromethorphan hydrobromide	1 mg/ml
Pseudoephedrine hydrochloride	20 µg/ml
Benzocaine	1.44 mg/ml
Menthol	5 mg/ml
Tobramycin	0.3 mg/ml
Mupirocin	2 mg/ml
Amoxicillin	1 mg/ml
Dexamethasone	1.53 µmol/l

All tested interfering substance concentrations showed no significant influence on the performance of the Infl A/B RT-PCR assay in combination with the EZ1 DSP Virus system.

Cross-contamination

The risk of cross contamination of the EZ1 DSP Virus system was analyzed by performing 9 runs on the EZ1 Advanced with alternating checkerboard patterns. To detect sample-to-sample carryover, the runs were performed with ParvoB19/CMV-positive plasma samples and ParvoB19/CMV-negative plasma samples in alternating positions. Every third run was performed using only negative plasma samples. All eluates were tested using a suitable CMV PCR assay as well as a suitable Parvo B19 PCR assay.

All of the ParvoB19/CMV-positive samples tested positive in PCR and all ParvoB19/CMV-negative samples tested negative. No cross-contamination was detected for a sample-to-sample or run-to-run carry over.

Performance Characteristics of EZ2 Connect MDx

Performance Characteristics for the EZ2 Connect MDx have been established in equivalency studies with the EZ1 Advanced XL using the EZ1 DSP Virus Kit. Kit-related performance characteristics like eluate stability or basic performance are valid for all instrument systems listed in the instruction for use of the EZ1 DSP Virus Kit since the kit as part of the system does not change for the different automated platforms.

Note: Performance Characteristics highly depend on various factors and relate to the specific downstream application. Performance has been established for the EZ1 DSP Virus Kit in conjunction with exemplary downstream applications. However, methods for isolating nucleic acids from biological specimen are used as a front-end for multiple downstream applications. Thus, performance parameters such as the influence of exogenous interfering substances, cross-contamination, or run precision need to be established for any such workflow as part of the downstream application development. It is therefore the responsibility of the user to validate the whole workflow to establish appropriate performance parameters.

Basic performance and compatibility to different downstream applications

Basic performance data generated using the EZ1 Advanced XL, EZ1 Advanced, or BioRobot EZ1 apply to the EZ2 Connect MDx instrument too (see page 2). Sample composition and the kit are identical for the instrument systems for use with the EZ1 DSP DNA Blood Kit. Furthermore, equivalency of the extraction procedures used on the EZ2 Connect MDx system was tested to show equal or improved basic performance of the system. During equivalency testing, compatibility to different downstream applications (including qPCR) was confirmed as well.

However, as only exemplary downstream methods were used, it is the responsibility of the user to validate the whole workflow within their specific application to establish appropriate performance parameters.

Freezing–thawing of samples

It is not recommended to refreeze thawed samples or store samples for over 6 hours at 2–8°C, as this leads to significantly reduced yields and quality of viral nucleic acids or bacterial DNA.

Precision

NA was extracted from 200 µl plasma sample spiked with HCV to a concentration of 1E+04 IU/ml and eluted in 150 µl. In total, 12 purification runs were performed with three different operators, on 3 different devices (per instrument type) and on 3 different days. The intra-run precision data are shown as standard deviations of the CT values (Figure 7).

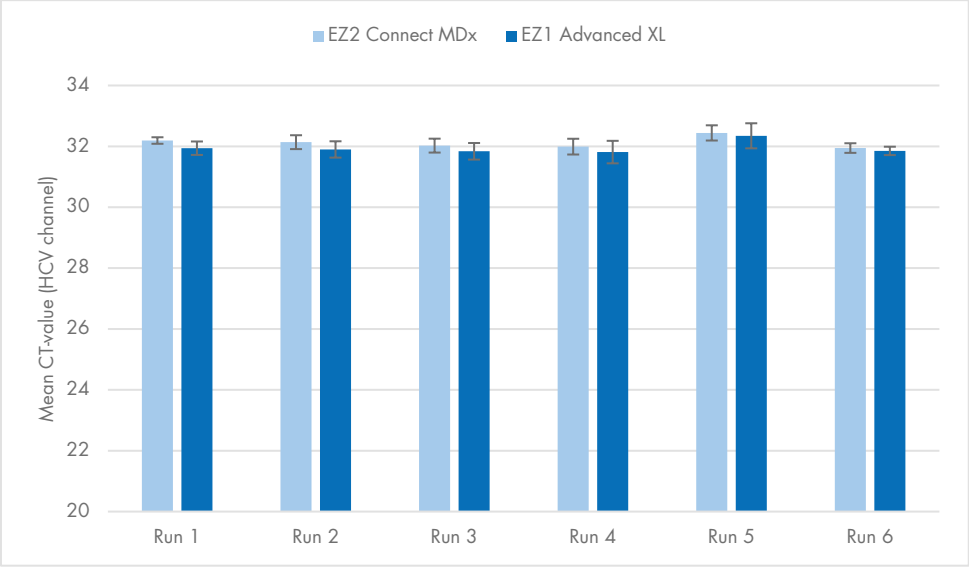

Figure 7. Mean Ct values of all runs using an HCV RT-PCR assay. Plasma was collected, pooled, and prepared with the respective virus titer before use. NA was purified from 200 µl aliquots in 6 runs of 12 replicates each on the EZ1 Advanced XL and the EZ2 Connect MDx using the EZ1 DSP Virus system. Mean CT values and standard deviations are shown for each run.

CVs were determined for the extraction of NA from plasma. The precision data are shown in Table 11.

Table 11. Analysis of precision estimates – intra-run variability

Precision	CV (%) (EZ2 Connect MDx)	CV (%) (EZ1 Advanced XL)
Intra run (Run 1)	0.33	0.69
Intra run (Run 2)	0.71	0.84
Intra run (Run 3)	0.71	0.86
Intra run (Run 4)	0.81	1.16
Intra run (Run 5)	0.77	1.27
Intra run (Run 6)	0.49	0.43

The intra-run variability for the EZ2 Connect MDx instrument was determined to be equivalent to the intra-run variability on the EZ1 Advanced XL instrument when using the EZ1 DSP Virus Kit in equivalency tests.

In addition, inter-run variability was determined for the EZ2 Connect MDx instrument (Table 12).

Table 12. Analysis of precision estimates – inter-run variability

Precision	CV (%) (EZ2 Connect MDx)	CV (%) (EZ1 Advanced XL)
Inter-run (Run 1–6)	0.82	1.06

The statistical analysis showed equal performance of the EZ2 Connect MDx compared to the EZ1 Advanced XL instrument.

Sample input/eluate output

The EZ1 DSP Virus system on the EZ2 Connect MDx offers the possibility to combine different sample input volumes (100, 200, or 400 µl) with different eluate output volumes (60, 90, 120, or 150 µl). Overall performance testing of the extraction procedures used on the EZ2 Connect MDx system showed equal performance of the system in relation to the EZ1 Advanced XL.

Depending on the complete workflow (sample preparation in combination with specific downstream application), there may be a most beneficial combination of sample input and elution volume that can help to optimize, for example, the final NA yield and concentration or to further minimize the potential influence of residual interfering substances. Different downstream applications even for the same sample material might require different sample input/eluate output combinations. Therefore, it is the user´s responsibility to validate the whole workflow within their specific application to establish appropriate performance parameters.

Sensitivity

Using plasma samples spiked with a HBV concentration close to the limit of detection (approx. 18 IU/ml), 18 purification runs on the EZ2 Connect MDx and EZ1 Advanced XL were performed by one operator on three different devices (per instrument type) on 3 days using 400 µl sample input and 90 µl elution volume. All eluates were subjected to qualitative analysis using a suitable HBV PCR assay whether or not target can be detected. Being close to the limit of detection, it is not expected that all replicates are determined to be positive. It could be confirmed though that the number of positive replicates is statistically equivalent.

Table 13. Summary of sensitivity test results from all E2 Connect MDx runs

	EZ2 Connect MDx – Hits of positive HBV samples								
No. of hits	8	8	7	7	7	8	8	6	7
% of hits	100%	100%	87.50%	87.50%	87.50%	100%	100%	75.00%	87.50%

Table 14. Summary of sensitivity test results from all EZ1 Advanced XL runs

	EZ1 Advanced XL – Hits of positive HBV samples								
No. of hits	8	8	8	7	7	8	8	7	7
% of hits	100%	100%	100%	87.50%	87.50%	100%	100%	87.50%	87.50%

Table 15. Sensitivity summary showing Fisher´s Exact Test results

EZ2 correct calls	EZ1 correct calls	Fisher´s Exact Test *P* value (2-Tail)
91.55%	94.44%	0.532

The statistical analysis showed equal performance of the EZ2 Connect MDx compared to the EZ1 Advanced XL instrument.

Eluate stability

Eluate stability data generated using the EZ1 Advanced XL, EZ1 Advanced, or BioRobot EZ1 apply to the EZ2 Connect MDx instrument too (see page 2). Sample and kit composition are identical for the instrument systems for use with the EZ1 DSP Virus Kit. Furthermore, equivalency of the extraction procedures used on the EZ2 Connect MDx system was tested to show equal performance of the system. The instructions for eluate handling apply to all automated systems for use with the kit.

However, it is the responsibility of the user to validate the whole workflow within their specific application to establish appropriate performance parameters.

Interfering substances

The influence of interfering substances was determined using the EZ1 Advanced XL. These data apply to the EZ2 Connect MDx instrument, too (see page 12). Sample and kit composition are identical for the instrument systems for use with the EZ1 DSP Virus Kit. The sample input/eluate output volumes are identical so that no impact on type or concentration of interfering substances in the eluates is expected. Furthermore, equivalency of the extraction procedures used on the EZ2 Connect MDx system was tested to show equal performance of the system. The instructions for sample and eluate handling apply to all automated systems for use with the kit.

However, it is the responsibility of the user to validate the whole workflow within their specific application to establish appropriate performance parameters.

Cross-contamination

The risk of cross contamination of the EZ1 DSP Virus Kit used on the EZ2 Connect MDx was analyzed by performing ten runs (400 µl input, 60 µl elution) with alternating checkerboard patterns on 2 days by one operator. To detect sample-to-sample carryover, the runs were performed with positive (spiked with HBV) and negative (un-spiked) plasma samples in alternating positions. Every second run was performed using only HBV-negative plasma samples. All eluates were analyzed using a suitable HBV PCR assay.

All HBV-positive samples tested positive in PCR and all HBV-negative plasma samples tested negative. No cross-contamination was detected for a sample-to-sample or run-to-run carry over.

Symbols

The following symbols appear in this document. For a full list of symbols used in the instructions for use or on the packaging and labeling, please refer to the handbook.

Symbol	Symbol definition
CE	This product fulfills the requirements of the European Regulation 2017/746 for in vitro diagnostic medical devices.
IVD	In vitro diagnostic medical device
REF	Catalog number
Rn	R is for revision of the Instructions for Use and n is the revision number
	Manufacturer
	Important note

Revision History

Revision	Description
R1, June 2022	Version 5, Revision 1 • Generation of document for new kit version. Data for EZ2 Connect MDx added • Removal of sample material whole blood, urine, dried swabs, sputum from intended use

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.