June 2022

QIAsymphony® DSP Virus/Pathogen Kit Instructions for Use (Performance Characteristics)

Version 2

IVD

For In Vitro Diagnostic Use
For use with QIAsymphony DSP Virus/Pathogen Mini and Midi Kits

CE

REF 937036, 937055

QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, Germany

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 QIAsymphony DSP Virus/Pathogen Kits are intended to be used only in combination with the QIAsymphony SP.

The QIAsymphony DSP Virus/Pathogen Kits provide reagents for fully automated and simultaneous purification of viral and bacterial nucleic acids. The kits can be used to purify nucleic acids from a broad range of DNA and RNA viruses as well as bacterial DNA from Gram-negative and Gram-positive bacteria. However, performance characteristics for every virus or bacteria species have not been established and must be validated by the user.

Magnetic-particle technology enables purification of high-quality nucleic acids that are free of proteins, nucleases, and other impurities. The purified nucleic acids are ready for direct use in downstream applications, such as amplification reactions (PCR). The QIAsymphony SP performs all steps of the purification procedure. Up to 96 samples, in batches of up to 24, are processed in a single run.

In the following selected performance data for the different applications are shown.

Performance Characteristics

Note: Performance Characteristics highly depend on various factors and relate to the specific downstream application. They have been established for the QIAsymphony DSP Virus/Pathogen 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. Performance parameters such as 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

Basic performance of the QIAsymphony DSP Virus/Pathogen Kit was evaluated, using HIV-1 RNA as an example virus. The tests were performed with dilutions of quantified virus panels made in HIV-1 negative human plasma. Dilution series with 7 different virus titers were tested with up to 6 replicates each, purified with the QIAsymphony DSP Virus/Pathogen Kit procedure, and analyzed for HIV-1 with an in-house RT-PCR assay (Figure 1). Viral nucleic acids were purified from 1000 µl samples with a 60 µl elution volume.

Furthermore, bacterial and viral nucleic acids and different qPCR downstream applications were utilized during kit development to demonstrate that the isolated nucleic acids are compatible with different downstream applications (Table 2–Table 7, Figure 2, and Figure 3).

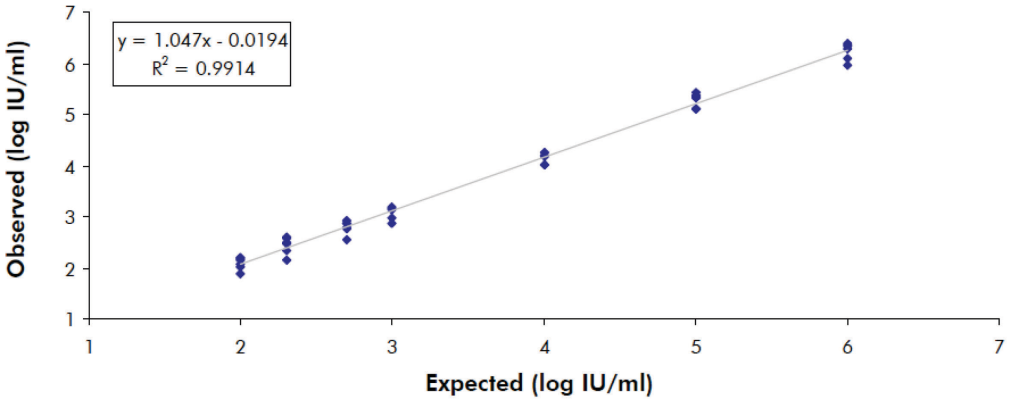

Figure 1. Observed yields using the Virus Cellfree 1000 protocol, with viral dilution series and an in-house RT-PCR assay for HIV-1 RNA virus.

Precision

Standard deviations and coefficients of variations (CVs) were determined for HIV-1 dilution series in the linear range of the appropriate downstream assays. For precision analysis, the same downstream assays were used as for determination of the basic performance (Figure 1). The inter-assay precision data are shown in Table 1. For each panel member, 5 or 6 replicates were extracted on the QIAsymphony SP.

Table 1. Inter-assay precision of the Virus Cellfree 1000 protocol using an in-house RT-PCR assay for HIV-1 RNA virus

Panel member	n	IU/ml	CV (%)	log IU/ml	SD (log IU/ml)
1	6	1 835 700	30.04	6.24	0.15
2	6	199 931	26.99	5.28	0.13
3	5	13 785	21.02	4.13	0.09
4	5	1363	17.49	3.13	0.09
5	6	642	24.82	2.79	0.12
6	6	294	31.12	2.44	0.16
7	6	123	23.25	2.08	0.11

Repeatability of the Complex 200, 400, and 800 protocols

Chlamydia trachomatis DNA was purified on the QIAsymphony SP from 200, 400, and 800 µl urine, and was eluted in 110 µl. For each protocol (Complex200_V5_DSP, Complex400_V3_DSP, and Complex800_V5_DSP), one operator performed 3 individual runs on the same instrument, on 3 different days, where each run consisted of 4 batches of 22 samples.

Table 2. Repeatability of the Complex 200 protocol using a *C. trachomatis* in-house assay

Run	Batch	*n*	Mean C_T	SD	CV (%)
1	1	22	28.74	0.32	1.10
	2	22	29.03	0.49	1.68
	3	22	29.00	0.53	1.84
	4	22	29.04	0.45	1.55
2	1	22	28.26	0.36	1.28
	2	22	28.90	0.27	0.93
	3	22	28.84	0.26	0.91
	4	22	28.94	0.31	1.08
3	1	22	27.87	0.39	1.40
	2	22	28.35	0.32	1.12
	3	22	28.52	0.28	0.97
	4	22	28.94	0.32	1.09
Total number of samples = 264					
Overall mean = 28.70					

Table 3. Precision of the Complex 200 protocol using a *C. trachomatis* in-house assay

	Batch-to-batch within the same run (S_{PWR})	Run to run (S_{BR})	Total (S_T)
SD	0.46	0.26	0.53
CV (%)	1.60	0.91	1.84

Table 4. Repeatability of the Complex 400 protocol using a *C. trachomatis* in-house assay

Run	Batch	n	Mean C_T	SD	CV (%)
1	1	22	27.32	0.43	1.57
	2	22	27.35	0.37	1.37
	3	22	27.54	0.44	1.61
	4	22	27.37	0.57	2.08
2	1	22	28.07	0.46	1.62
	2	22	28.42	0.55	1.93
	3	22	28.47	0.55	1.95
	4	22	28.61	0.32	1.11
3	1	22	27.85	0.53	1.89
	2	22	28.60	0.44	1.53
	3	22	28.09	0.87	3.11
	4	22	28.23	0.35	1.24
Total number of samples = 264					
Overall mean = 27.99					

Table 5. Precision of the Complex 400 protocol using a *C. trachomatis* in-house assay

	Batch-to-batch within the same run (S_{PWR})	Run to run (S_{BR})	Total (S_T)
SD	0.51	0.52	0.73
CV (%)	1.83	1.87	2.62

Table 6. Repeatability of the Complex 800 protocol using a *C. trachomatis* in-house assay

Run	Batch	n	Mean C_T	SD	CV (%)
1	1	22	26.04	0.34	1.32
	2	22	26.07	0.43	1.66
	3	22	26.81	0.47	1.76
	4	22	26.10	0.41	1.59
2	1	22	26.17	0.29	1.10
	2	22	26.35	0.43	1.65
	3	22	26.11	0.34	1.31
	4	22	26.15	0.37	1.41
3	1	22	26.05	0.33	1.25
	2	22	26.32	0.54	2.04
	3	22	25.72	0.41	1.60
	4	22	26.59	0.48	1.81
Total number of samples = 264					
Overall mean = 26.20					

Table 7. Precision of the Complex 800 protocol using a *C. trachomatis* in-house assay

	Batch-to-batch within the same run (S_{PWR})	Run to run (S_{BR})	Total (S_T)
SD	0.46	0.00	1.76
CV (%)	0.46	0.00	1.76

Eluate stability

Note: Eluate stability highly depends on various factors and relates to the specific downstream application. It has been established for the QIAsymphony DSP Virus/Pathogen Kit in conjunction with exemplary downstream applications. It is the responsibility of the user to consult the instructions for use of the specific downstream application used in their laboratory and/or validate the whole workflow to establish appropriate storage conditions.

Eluate stability for the QIAsymphony DSP Virus/Pathogen Kit was evaluated, using extracted nucleic acid from urine spiked with HIV standard material and CMV standard material. Stability of the nucleic acid was determined with in-house real-time PCR assays for HIV and CMV. Eluate stability at 2–8°C was not affected by duration of the storage up to 1 month. However, for storage times of over 24 hours, we recommend to store purified nucleic acids at –20°C.

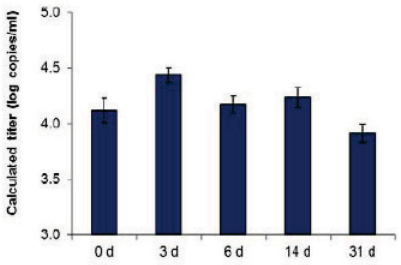

Figure 2. Stability of HIV RNA in eluates. HIV standard material spiked in urine was purified on the QIAsymphony SP using the Complex 200 protocol. Eluates were incubated for 31 days at 2–8°C. An in-house real-time PCR assay for HIV was used for detection at regular timepoints. Eluates were analyzed in replicates of 8.

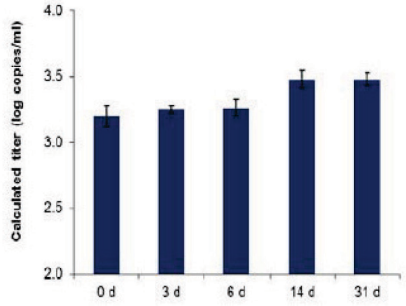

Figure 3. Stability of CMV in eluates. CMV standard material spiked in urine was purified on the QIAsymphony SP using the Complex 200 protocol. Eluates were incubated for 31 days at 2–8°C. An in-house real-time PCR assay for CMV was used for detection at regular timepoints. Eluates were analyzed in replicates of 8.

Interfering substances

Different potential endogenous and exogenous interferents were spiked in EDTA plasma, CSF, urine, and transport medium (eNAT) with virus material to test their impact on exemplary downstream assays after sample preparation with the QIAsymphony DSP Virus/Pathogen Kit. Common relevant potential interferents and the respective tested sample materials are listed below in Table 8. No significant negative impact was observed for the listed interferents and over 80 additional potential interferents.

Table 8. Potential interfering substances tested with different sample materials

Interfering substances	Plasma	CSF	Urine	eNAT
(Human Serum) Albumin	√		√	
Bilirubin	√		√	
Erythrocytes		√	√	
Gamma Globulin	√			
gDNA	√	√	√	
Hemoglobin	√			
Human Liver Total RNA	√			
Triglyceride (Intralipid)	√			
EDTA	√			
Heparin	√			
Ammonia solution	√			
Glucose			√	
Mucus			√	√
Blood			√	√
Leukocytes			√	√
pH 4, pH 9			√	

Note: "√" indicates which sample materials were tested for the respective potential interfering substance.

Any potential interfering substances (e.g., drugs) and corresponding concentration are very specific to the downstream application and possible previous medical treatments of a patient and needs to be investigated during verification of such downstream application using the QIAsymphony DSP Virus/Pathogen Kits.

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 or concentration of potential interfering substances), so the identification and testing of relevant substances and respective concentrations also need to be established as part of the downstream application development for any workflow involving the QIAsymphony DSP Virus/Pathogen Kits.

Note: According to ISO 20186-2:2019(E), heparin from blood collection tubes may impact the purity of the isolated nucleic acids and possible carryover into eluates could cause inhibitions in some downstream applications. Therefore, we recommend usage of blood samples treated with EDTA or citrate as anticoagulant for plasma preparation.

Cross-contamination

The risk of cross-contamination of the QIAsymphony DSP Virus/Pathogen Kits was analyzed by performing three 96 sample runs on the QIAsymphony SP instrument with alternating checkerboard batches (positive and negative samples alternating). Human EDTA plasma and urine spiked with HIV material (2.93E+07 and >1.00E+07 IU/ml, respectively) were used as a model system. Sample preparation was performed using all available protocols (for Virus Cellfree and Pathogen Complex applications). A potential contamination of the negative plasma and urine samples during the extraction runs was evaluated by subsequent analysis of the eluates using an in-house RT-PCR assay for HIV virus. No cross-contamination was detected for sample-to-sample, batch-to-batch, or run-to-run carry over.

Sample input/eluate output range

Different sample input and elution volumes can be selected for sample preparation using the QIAsymphony DSP Virus/Pathogen Kits. For further details, see the protocol sheets that can be found under the resource tab of the product page on **www.qiagen.com**. Exemplary correlation studies have been performed for EDTA plasma spiked with HBV and HIV virus material using the Cellfree 200 and Cellfree 1000 protocols to analyze the influence of the three different elution volumes. The results show no significant differences in the quantification of an RNA or DNA virus using the Cellfree 200 or Cellfree 1000 protocol in combination with one of the three different elution volumes (60, 85, and 110 µl).

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

Revision History

Revision	Description
R1, June 2022	Version 2, Revision 1 • Update to version 2 for compliance to IVDR • Transfer of Linear range section into Basic performance and compatibility to different downstream applications section • Extension of Eluate stability section • Addition of Interfering substances section • Addition of Cross-contamination section • Addition of Sample input/eluate output range section • Addition of Symbols section

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.