June 2022

QIAamp® DSP Virus Spin Kit Instructions for Use (Performance Characteristics)

Version 2

IVD

For In Vitro Diagnostic Use

For use with QIAamp® DSP Virus Spin Kit

CE

REF 61704

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R1

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

Sample to Insight

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General introduction

The QIAamp® DSP Virus Spin Kit is intended for manual or, when used in conjunction with the QIAcube® Connect MDx instrument, for automated isolation and purification of viral nucleic acids from human plasma and serum samples. The QIAamp DSP Virus Spin Kit utilizes silica-membrane technology (QIAamp technology) for isolation and purification of viral nucleic acids from human plasma and serum samples.

The QIAamp DSP Virus Spin procedure comprises 4 steps (lyse, bind, wash, and elute) and is carried out using QIAamp MinElute® columns in a standard microcentrifuge or automated on the QIAcube Connect MDx. The procedure is designed to minimize the potential for sample-to-sample cross-contamination and allows safe handling of potentially infectious samples. The simple QIAamp DSP Virus Spin procedure is suitable for simultaneous processing of multiple samples. The QIAamp DSP Virus Spin Kit can be used for isolation of viral RNA and DNA from a broad range of RNA and DNA viruses.

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

Performance Characteristics

Note: Performance Characteristics highly depend on various factors and relates to the virus species and the specific downstream application. Performance characteristics have been established for the QIAamp DSP Virus Spin Kit in conjunction with exemplary virus species and exemplary downstream applications. However, methods for isolating nucleic acids from biological specimen are used as a front-end for multiple downstream applications. Performance parameter e.g. 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.

Kit performance is not guaranteed for each virus 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.

Basic Performance and compatibility to different downstream applications

The performance for automated purification of viral nucleic acid using the QIAamp DSP Virus Spin Kit was analyzed with human plasma and serum and hepatitis C virus (HCV) RNA as an example virus. The tests were performed with a dilution of quantified virus panels made in HCV-negative human plasma and serum (n=15). HCV RNA was detected using a real-time PCR assay (Figure 1). Viral nucleic acids were purified from 200 µl samples with the standard and the manual lysis protocol and an elution volume of 60 µl.

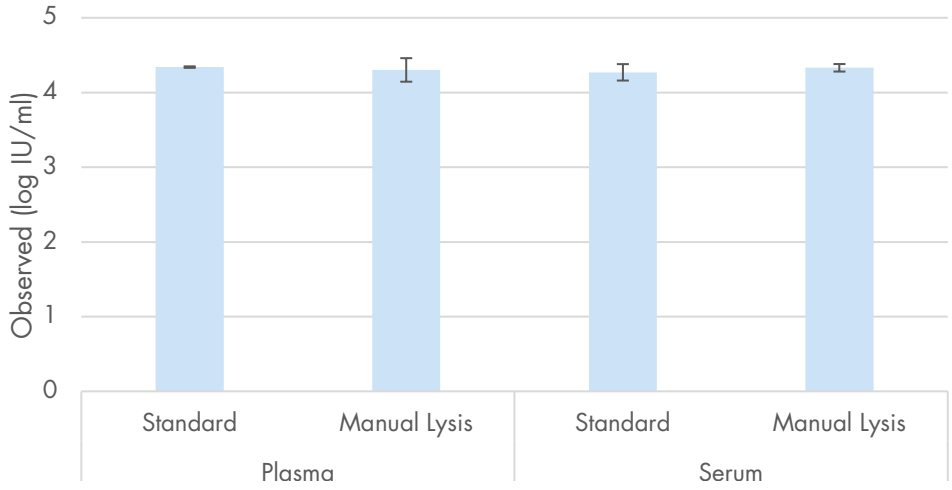

Figure 1 . Performance of the automated purification of viral nucleic acid using the QIAamp DSP Virus Spin Kit. The performance of the QIAamp DSP Virus Spin Kit in two different protocols (standard and manual lysis) was analyzed with serum and plasma samples. Viral RNA was detected using viral dilution series and a real-time PCR assay for HCV RNA.

Furthermore, performance for automated and manual extraction of Hepatitis C virus (HCV) RNA using the QIAamp DSP Virus Spin Kit was tested with dilution series of quantified virus panels made in HCV-negative human plasma. Dilution series with 5 different virus titers were tested, with 12 replicates each. HCV RNA was detected using an real-time PCR assay (Figure 2). Viral nucleic acids were purified from 200 µl samples with a 60 µl elution volume.

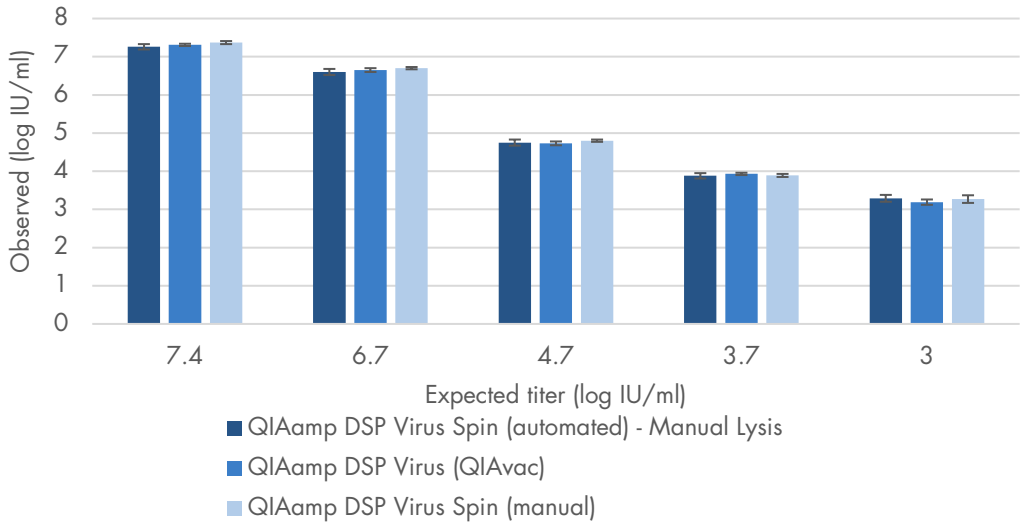

Figure 2. Virus titers determined by exemplary real-time PCR assay for HCV after using the QIAamp DSP Virus Spin Kit for manual and automated purification of viral dilution series of HCV from human plasma and an elution volume of 60 µl.

Besides, further exemplary 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 (see sections below and Table 1).

Sample Input/Eluate Output Range

The starting sample volume for purification of viral nucleic acids from human plasma and serum samples using the QIAamp DSP Virus Spin Kit is 200 µl. For the manual spin workflow flexible elution volumes between 20 and 150 µl can be selected. For the automated spin workflow on the QIAcube Connect MDx, elution volumes of 60–100 µl in 5 µl increments can be selected.

Different eluate volumes were analyzed with various exemplary downstream real-time PCR assays for HBV, HCV, and HIV using the QIAamp DSP Virus Spin Kit.

Precision

Coefficients of variations (CVs) were determined using the QIAamp DSP Virus Spin Kit on the QIAcube Connect MDx for automated extraction of viral nucleic acid from human EDTA plasma spiked with HBV and HCV standard material (2.5E+03 IU/ml for both). Virus titers were determined using real-time PCR assays for HBV and HCV.

Repeatability (intra-run variability within one purification run) and total precision were determined. The precision data are shown in Table 1. For precision analysis, total DNA yield was determined by OD-measurement.

Table 1. Analysis of precision estimates

Assay	Precision	CV (%)
HBV	Repeatability	0.79
	Total precision	0.90
HCV	Repeatability	0.57
	Total precision	0.59

Eluate stability

Note: Eluate stability highly depends on various factors and relates to the specific downstream application. It has been evaluated for isolation of viral nucleic acid using with the QIAamp DSP Virus Kit, that uses identical chemistry 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 QIAamp DSP Virus Kit was evaluated using 500 µl EDTA plasma samples spiked with HBV and HCV standard material ($1x10^4$ IU/ml for both) and a 60 µl elution volume. Stability of the nucleic acid was determined with real-time PCR assays for HBV and HCV. Eluate stability at 2–8°C was not affected by duration of the storage up to 2 weeks. However, for storage times of over 24 hours we recommend to store purified nucleic acids for up to 6 months at –20°C and for up to 12 months at –80°C.

Interfering substances

Different potential exogenous and endogenous interfering substances present in patient blood were spiked into EDTA plasma with virus standard material to test their impact on exemplary downstream assays after automated purification of viral nucleic acids with the QIAamp DSP Virus Spin Kit and the QIAamp DSP Virus Kit, that uses identical chemistry.

Common relevant potential interfering substances for haemolysis (human hemoglobin), lipemia (triglycerides), and jaundice (bilirubin unconjugated) were evaluated in exemplary downstream assays. No significant negative impact was observed for these potential interferents and for over 30 additional potential interferents such as drugs typically used e.g. for treatment of relevant viral infections or other opportunistic infections and, thus, likely to be found in patient samples.

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 concentration also needs to be established as part of the downstream application development for any workflow involving the QIAamp DSP Virus/Virus Spin Kit.

However, interference could be detected in a real-time PCR assay for heparinized plasma. This is in line with ISO 20186-2:2019(E), that suggests that heparin from blood collection tubes may impact the purity of the isolated nucleic acids and possible carryover into eluates may cause inhibitions in some downstream applications. Therefore, we recommend usage of blood samples treated with EDTA or citrate as anticoagulant for plasma preparation.

Any potential interfering substances (e.g. drugs) and corresponding concentration is 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 QIAamp DSP Virus/Virus Spin Kit.

Cross contamination

The risk of cross contamination for the automated purification of viral nucleic acid using the QIAamp DSP Virus Spin Kit was analyzed by performing five 12 sample runs with alternating checker board batches (positive and negative samples alternating) for plasma and serum samples spiked with 1.00E+07 copies/ml HBV virus. A potential contamination of the negative samples during the extraction runs was evaluated by subsequent analysis of the eluates using real time-PCR assay. No cross-contamination was detected for 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
	Consult instructions for use
	Important note

Document Revision History

Revision	Description
R1, June 2022	Version 2, Revision 1 ● Update to Version 2 for compliance to IVDR ● Transfer and update of performance characteristics from kit handbook to this document ● Addition of the following sections: ○ Basic Performance and compatibility to different downstream applications ○ Sample Input/Eluate Output Range ○ Precision ○ Addition Interfering Substances ○ Cross contamination ○ Symbols ○ Document Revision History

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