QIAsymphony® DSP Virus/Pathogen Kit

QIAsymphony DSP Virus/Pathogen Kits are intended to be used only in combination with the QIAsymphony SP. QIAsymphony DSP Virus/Pathogen Kits provide reagents for fully automated and simultaneous purification of viral nucleic acids from serum, plasma, or CSF or of viral nucleic acids and bacterial DNA from various materials, including respiratory samples such as swabs, aspirates, sputum, bronchoalveolar lavage (BAL), as well as urine and urogenital swabs (cervical and urethral). 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, kit performance is not guaranteed for each virus or bacteria species and must be validated by the user.

Performance characteristics

Linear range

The linear range for 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. The linear range of the QIAsymphony DSP Virus/Pathogen Kit procedure has been determined 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.

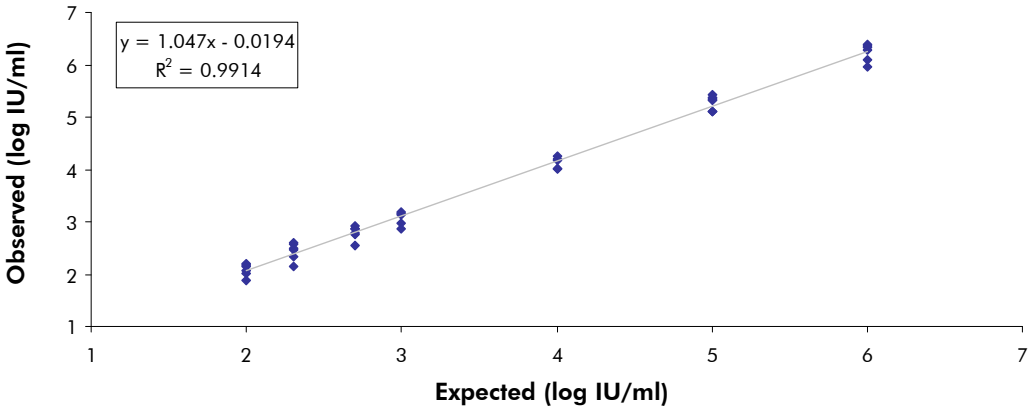

Figure 1. Linear range of yields using the Virus Cellfree 1000 protocol. The linear range of the Virus Cellfree protocol was determined using viral dilution series and an in-house RT-PCR assay for HIV-1 RNA virus.

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 linear range (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	1835700	30.04	6.24	0.15
2	6	199931	26.99	5.28	0.13
3	5	13785	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 1. Repeatability of the Complex 200 protocol using a *C. trachomatis* in-house assay

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

Table 2. 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_r)
SD	0.46	0.26	0.53
CV (%)	1.60	0.91	1.84

Table 3. Repeatability of the Complex 400 protocol using a *C. trachomatis* in-house assay

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

Table 4. 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_r)
SD	0.51	0.52	0.73
CV (%)	1.83	1.87	2.62

Table 3. Repeatability of the Complex 800 protocol using a *C. trachomatis* in-house assay

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

Table 4. 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_r)
SD	0.46	0.00	1.76
CV (%)	0.46	0.00	1.76

Pretreatment of viscous samples and lysis of Gram-positive bacteria

Sputum samples were spiked with defined volumes of *Mycobacterium tuberculosis* culture suspension. Samples were combined with Sputasol in a 1:1 ratio to liquefy them, and then incubated at 37˚C for 30 minutes. Aliquots (1 ml) of liquefied sample were centrifuged at 5000 x g for 10 minutes. Pellets were resuspended in lysozyme solution (500 µl) and incubated at 37°C for 30 minutes. Three lysozyme solutions, each containing 1 of 3 different lots of lysozyme, were used. *M. tuberculosis* DNA was purified from these lysozyme-treated samples (200 µl) using the Complex 200 protocol on the QIAsymphony SP or manually using the QIAamp DNA Mini Kit. Eluates were analyzed using an in-house real-time PCR assay specific for *M. tuberculosis*.

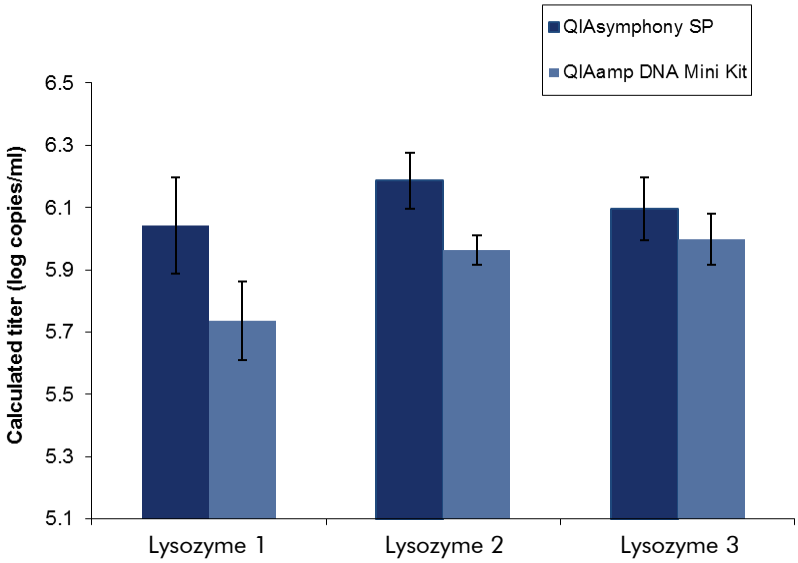

Figure 2. Pretreatment of viscous samples and lysis of Gram-positive bacteria.

Eluate stability

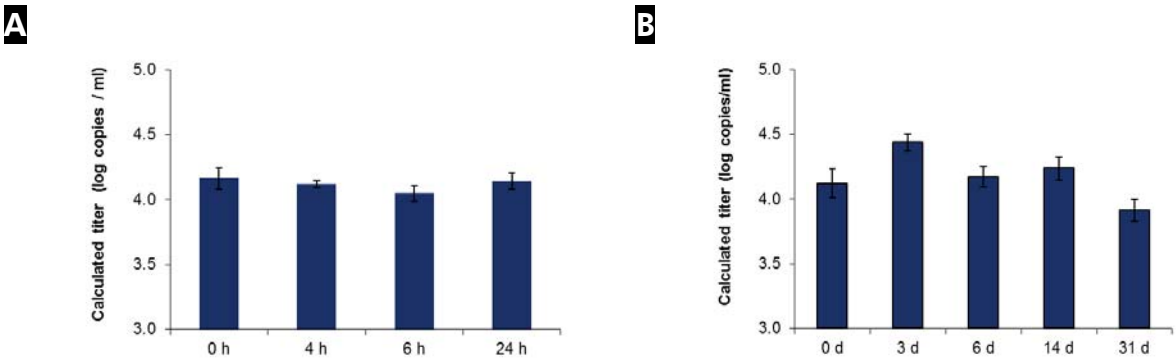

Figure 3. Stability of HIV RNA in eluates. HIV standard material spiked in urine was purified on the QIAsymphony SP using the Complex 200 protocol. Eluates were incubated **A** for 24 hours at

37°C, and **B** for 31 days at 5°C. An in-house real-time PCR assay for HIV was used for detection at regular timepoints. Eluates were analyzed in replicates of 8.

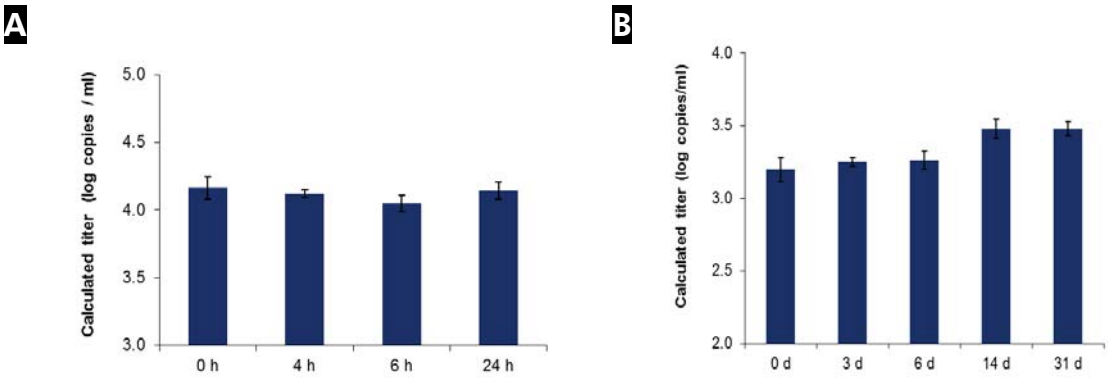

Figure 4. Stability of CMV in eluates. CMV standard material spiked in urine was purified on the QIAsymphony SP using the Complex 200 protocol. Eluates were incubated **A** for 24 hours at 37°C and **B** for 31 days at 5°C. An in-house real-time PCR assay for CMV was used for detection at regular timepoints. Eluates were analyzed in replicates of 8.

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.

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