August 2015

QIAsymphony® DSP DNA Kits: Performance Characteristics

This document is *QIAsymphony DSP DNA Kits: Performance Characteristics*, R4, for Kit Version 1

Sample to Insight

QIAGEN

QIAsymphony DSP DNA Kits are intended to be used only in combination with the QIAsymphony SP. QIAsymphony DSP DNA Mini Kits provide reagents for automated purification of total DNA from human whole blood, buffy coat, tissue and formalin-fixed, paraffin-embedded tissue samples, as well as viral DNA from human whole blood. QIAsymphony DSP DNA Midi Kits provide reagents for automated purification of total DNA from human whole blood and buffy coat.

Tissue and FFPE tissue

Linear range

The linear range for the QIAsymphony DSP DNA FFPE tissue application was evaluated using six replicates of 1–4, 10 µm FFPE sections of freshly cut human spleen. DNA extraction was performed using the QIAsymphony DSP DNA Mini Kit in combination with the tissue low content DSP protocol. Deparaffinization and lysis were performed using the xylene/ethanol pretreatment method. DNA was eluted in 50 µl elution buffer and the yield of DNA was determined by spectroscopic analysis (Figure 1).

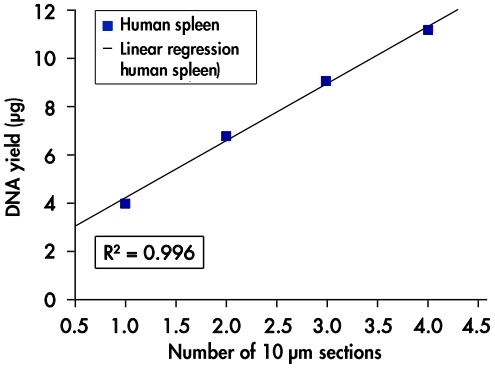

Figure 1. Linear range of DNA extracted from FFPE tissue sections. Six replicates of 1–4, 10 µm FFPE tissue sections of human spleen were deparaffinized by xylene/ethanol pretreatment. DNA extraction was performed on the QIAsymphony SP using the QIAsymphony DSP DNA Mini Kit in combination with the tissue low content DSP protocol and an elution volume of 50 µl.

Comparative performance

The performance of the QIAsymphony DSP DNA Mini Kit was compared to the performance of the manual QIAamp® DSP DNA FFPE Tissue Kit and the QIAamp DSP DNA Mini Kit using FFPE tissue and fresh/frozen tissue, respectively, as sample material. Manual and automated sample preparations, as well as quantification of the DNA yields, were performed simultaneously. DNA yields after extraction from fresh/frozen and FFPE tissue samples using the QIAsymphony DSP DNA Mini Kit, QIAamp DSP DNA Mini Kit (Tissue) and the QIAamp DSP DNA FFPE Tissue Kit (FFPE tissue) are shown in Figure 2.

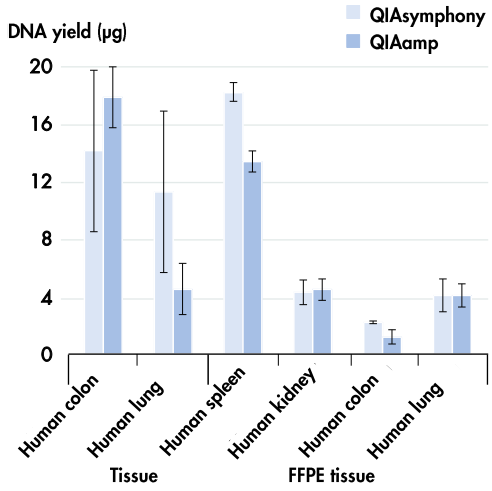

Figure 2. DNA extraction from tissue and FFPE tissue samples. For fresh/frozen tissue, human lung and colon samples were cut into 6 x 25 mg pieces. Three pieces of each tissue type were used for sample preparation using the QIAsymphony SP in combination with the tissue high content DSP protocol. DNA extraction from remaining samples was performed using the QIAamp DSP DNA Mini Kit. DNA was eluted in 200 µl and DNA yield was determined by spectroscopic analysis. For DNA extraction from FFPE tissue, 12 replicates containing 3 x 10 µm FFPE tissue sections from various human organs were prepared. Six samples were used for sample preparation using the QIAsymphony SP in combination with the Deparaffinization Solution pretreatment and the tissue low content DSP protocol. DNA extraction from remaining samples was performed using the QIAamp DSP DNA FFPE Tissue Kit. DNA was eluted in 50 µl and DNA yield was determined by spectroscopic analysis. Bars show the absolute DNA yield with standard deviation.

Analysis of mutational status of biomarkers by real-time PCR

Analysis of mutational status of biomarkers was performed using DNA extracted from FFPE sections of human colon and DNA extracted from human lung tissue samples.

For DNA extraction from FFPE tissue samples, 3 x 10 µm sections of human colon were used for sample preparation. DNA extraction was performed using Deparaffinization Solution for pretreatment and the tissue low content DSP protocol in combination with the 100 µl elution volume. Mutational analysis of biomarker KRAS was performed using the KRAS RGQ PCR Kit in accordance with the kit handbook. C_T values of the control assay were within the defined range and mutation detection analysis revealed an amino acid substitution in codon 12 (Table 1, page 4).

For DNA extraction from frozen tissue samples, 25 mg of human lung was used for sample preparation using the tissue high content DSP protocol and an elution volume of 200 µl. Mutational analysis of the EGFR biomarker was performed. Control and mutation detection assays were performed as described in the therascreen® *EGFR RGQ PCR Kit Handbook*. Results revealed a deletion within the EGFR gene, as demonstrated by a ΔC_T value of 2.47, which is below the defined cut-off value of 12 for the detection of a mutation (Table 2, page 5).

Table 1. Results of FFPE tissue KRAS biomarker mutational analysis

Sample	Reaction	Target C_T	Internal control C_T	ΔC_T*
No template control	Control	0.00	32.75	–
	12ALA	0.00	32.65	–
	12ASP	0.00	32.69	–
	12ARG	0.00	32.86	–
	12CYS	0.00	32.35	–
	12SER	0.00	32.76	–
	12VAL	0.00	32.41	–
	13ASP	0.00	32.26	–
Standard	Control	25.95	32.73	–
	12ALA	26.39	32.29	0.44
	12ASP	26.54	32.15	0.59
	12ARG	26.35	32.14	0.40
	12CYS	26.31	32.47	0.36
	12SER	26.50	32.34	0.55
	12VAL	25.80	31.92	–0.15
	13ASP	27.09	32.54	1.14
FFPE tissue	Control	24.94	31.98	–
(human colon)	12ALA	n.d.	32.42	–
	12ASP	n.d.	32.73	–
	12ARG	n.d.	33.05	–
	12CYS	n.d.	32.74	–
	12SER	29.11	32.34	4.17
	12VAL	n.d.	32.81	–
	13ASP	n.d.	33.20	–

* ΔC_T = M C_T – C C_T, where M = mutation and C = control; n.d. = not detected.

Table 2. Results of frozen tissue EGFR biomarker mutational analysis

Sample	Reaction	Target C_T	Internal control C_T	ΔC_T*
No template control	Control	0.00	31.71	–
	T790M	0.00	32.36	–
	Deletions	0.00	31.75	–
	L858R	0.00	32.05	–
	L861Q	0.00	31.77	–
	G719X	0.00	31.68	–
	S768I	0.00	32.25	–
	Ins	0.00	31.84	–
Standard	Control	28.78	31.05	–
	T790M	30.08	31.13	1.30
	Deletions	28.23	31.19	–0.55
	L858R	27.58	30.83	–1.20
	L861Q	27.80	30.86	–0.98
	G719X	27.80	30.90	–0.98
	S768I	29.28	31.41	0.50
	Ins	28.00	31.64	–0.78
Tissue (human lung)	Control	25.76	31.23	–
	T790M	n.d.	31.99	–
	Deletions	28.23	30.99	2.47
	L858R	n.d.	31.33	–
	L861Q	n.d.	31.98	–
	G719X	n.d.	32.06	–
	S768I	n.d.	31.88	–
	Ins	n.d.	31.62	–

* ΔC_T = M C_T – C C_T, where M = mutation and C = control; n.d. = not detected.

Blood and buffy coat

Performance characteristics for blood and buffy coat applications were performed using samples from blood donors with a white blood cell count range from 4.0 to 11.0 x 10^6 cells/ml and buffy coat donors with a white blood cell count range from 2.5 to 5.5 x 10^7 cells/ml.

DNA yield and purity

Basic performance of the QIAsymphony DSP DNA Mini Kit was evaluated using different collection tubes and anticoagulants, as well as fresh and frozen human whole blood. Whole blood was collected from 3 healthy donors in 3 different types of tubes: EDTA = BD™ 10 ml Vacutainer® 16 x 100 mm, K2-EDTA; Citrate = BD 2.7 ml 9NC Tube 13 x 75 mm, Citrate; Heparin = Sarstedt® 7.5 ml S-Monovette® 15 x 92 mm, Li-Heparin. Blood was used either fresh (stored at 5°C) or frozen (stored at –20°C). Genomic DNA was purified from 200 µl samples, with 4 replicates per donor and tube type, using the QIAsymphony DSP DNA Mini Kit and

blood 200 DSP protocol with an elution volume of 200 µl. DNA yields and purity were determined by spectroscopic analysis (Figure 3).

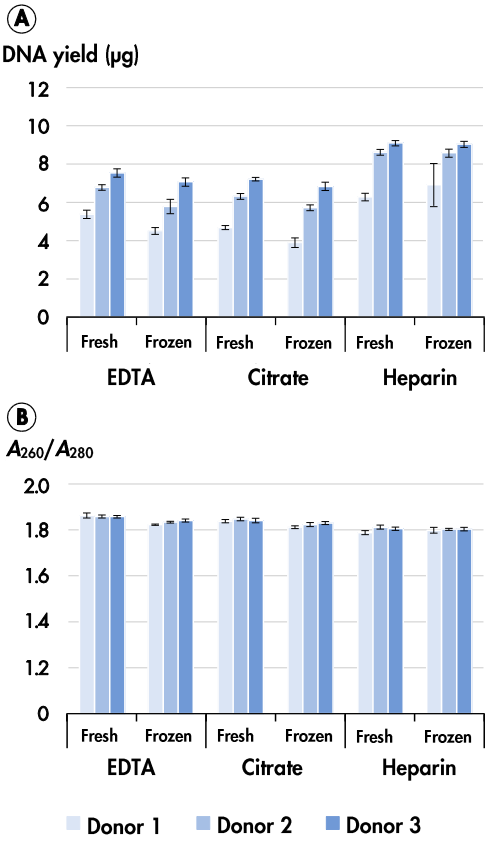

Figure 3. System robustness using different sample collection tubes and anticoagulants with fresh and frozen human whole blood. **A** DNA yield, bars show the absolute DNA yield with standard deviation. **B** DNA purity, bars show the DNA purity with standard deviation.

DNA integrity

Long-range PCR products (5 kb) were amplified using the QIAGEN® LongRange PCR Kit (50 µl reaction) (Figure 4, page 7).

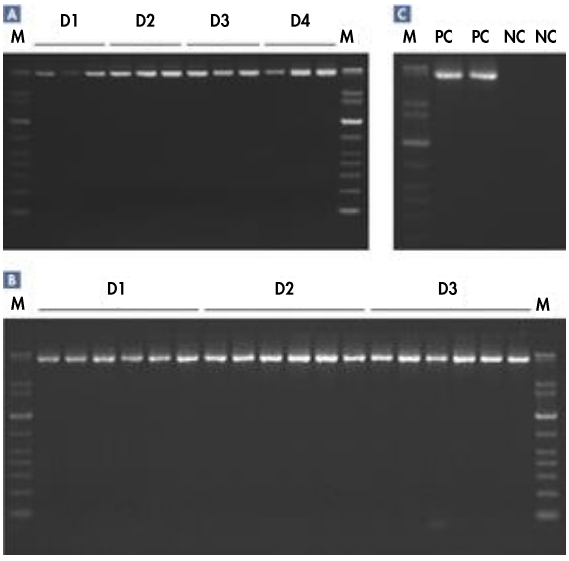

Figure 4. **DNA integrity tested by long-range PCR**. M = QIAGEN GelPilot 1 kb Plus Ladder. **A** Whole blood was collected from 4 healthy donors (D) in BD K2E tubes. Genomic DNA for long-range PCR was purified from 200 µl aliquots in triplicate using the QIAsymphony DSP DNA Mini Kit and blood 200 DSP protocol with an elution volume of 200 µl. D1 = Donor 1, D2 = Donor 2, D3 = Donor 3, and D4 = Donor 4. **B** Whole blood was collected from 3 healthy donors in BD K2E tubes and buffy coat was prepared. Genomic DNA was purified from 200 µl aliquots in six replicates using the QIAsymphony DSP DNA Mini Kit and buffy coat 200 DSP protocol with an elution volume of 200 µl. D1 = Donor 1, D2 = Donor 2, and D3 = Donor 3. **C** Controls: PC = positive control and NC = negative control.

Repeatability and reproducibility

DNA extraction was performed using the blood 200 DSP protocol with an elution volume of 200 µl. Repeatability was evaluated by a single operator performing three independent runs (96 samples each) on three different days, with each run consisting of 4 batches of 24 samples (Tables 3 and 4, page 8).

Reproducibility was evaluated by performing three independent runs (96 samples each) on three different days, by three different operators on different QIAsymphony SP instruments, with each run consisting of 4 batches of 24 samples (Tables 5 and 6, pages 8 and 9).

Table 3. Results of repeatability evaluation

Run	Batch	N	Mean DNA yield (µg)	SD	CV
1	1	24	5.32	0.22	4.22
	2	24	4.90	0.22	4.54
	3	24	4.95	0.21	4.26
	4	24	5.05	0.18	3.60
2	1	24	5.17	0.30	5.84
	2	24	4.90	0.15	3.14
	3	24	4.82	0.20	4.13
	4	24	4.87	0.17	3.52
3	1	24	5.11	0.17	3.33
	2	24	4.84	0.24	4.91
	3	24	4.87	0.16	3.38
	4	24	4.78	0.16	3.38
Total	–	288	4.96	–	–

* N = Number of replicates; SD = Standard deviation; CV = Coefficient of variation.

Table 4. Precision data for repeatability evaluation

	SD	CV
Batch to batch within same run	0.25	4.95
Overall repetition accuracy	0.26	5.18

* SD = Standard deviation; CV = Coefficient of variation.

Table 5. Results of reproducibility evaluation

Run	Batch	N	Mean DNA yield (µg)	SD	CV
1	1	24	5.32	0.22	4.22
	2	24	4.90	0.22	4.54
	3	24	4.95	0.21	4.26
	4	24	5.05	0.18	3.60
2	1	24	5.73	0.22	3.81
	2	24	5.56	0.26	4.63
	3	24	5.40	0.20	3.63
	4	24	5.46	0.21	3.89
3	1	24	5.73	0.26	4.62
	2	24	5.54	0.24	4.40
	3	24	5.41	0.18	3.34
	4	24	5.49	0.17	3.16
Total	–	288	5.38	–	–

* N = Number of replicates; SD = Standard deviation; CV = Coefficient of variation.

Table 6. Precision data for reproducibility evaluation

	SD	CV
Batch to batch within same run	0.25	4.73
Overall repetition accuracy	0.38	7.03

* SD = Standard deviation; CV = Coefficient of variation.

Linear range

The linear ranges for the QIAsymphony DSP DNA Blood and buffy coat applications were evaluated using blood and buffy coat samples with six different white blood cell (WBC) counts for each sample type. For whole blood, WBC counts ranged from 4 x 10^6 cells/ml to 11.6 x 10^6 cells/ml and for buffy coat, counts ranged from 2.2 x 10^7 cells/ml to 5.6 x 10^7 cells/ml. DNA yields were determined by spectroscopic analysis and plotted against the WBC count (Figure 5, page 10).

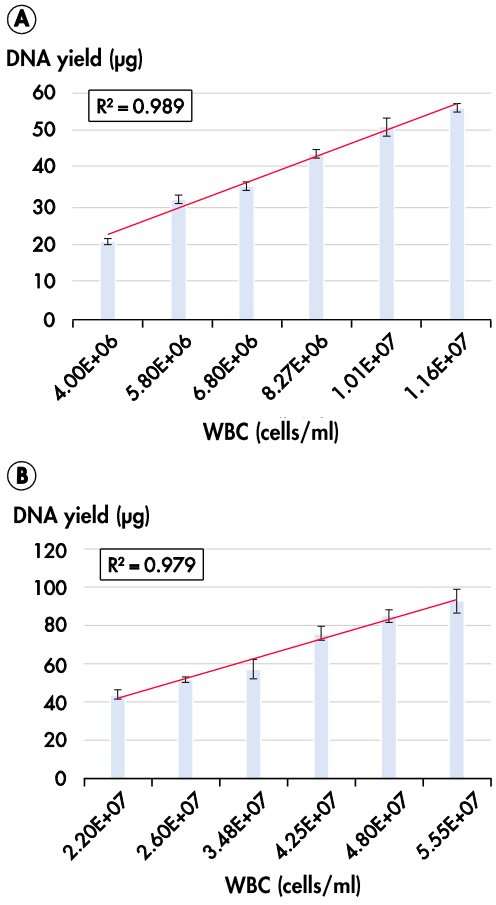

Figure 5. Linear range of DNA extracted from blood and buffy coat. A Genomic DNA was purified from 1 ml human whole blood using the QIAsymphony DSP DNA Midi Kit and the blood 1000 DSP protocol with an elution volume of 500 µl. Bars show the absolute DNA yield with standard deviation. B Genomic DNA was purified from 400 µl buffy coat using the QIAsymphony DSP DNA Midi Kit and the buffy coat 400 DSP protocol with an elution volume of 400 µl. Bars show the absolute DNA yield with standard deviation.

Comparative performance

Performance was analyzed for the QIAsymphony DSP DNA blood system in comparison to the EZ1® DSP DNA blood system and the QIAamp® DNA Blood Mini Kit manual preparation procedure. DNA was purified from different blood samples, analyzed for DNA yield (Figure 6, page 11) and used in the CE-marked *artus*® MTHFR LC PCR Kit (24) CE analysis (Table 7, pages 11 and 12).

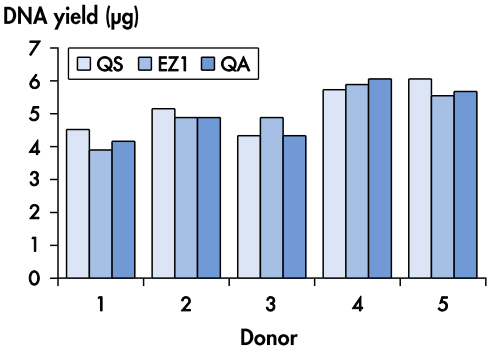

Figure 6. Comparison of DNA yields between different blood DNA purification systems. Whole blood was collected from 5 healthy donors in BD K2E tubes. For all methods, 200 µl sample input volumes and elution volumes of 200 µl were used. QS = QIAsymphony DSP DNA Mini Kit and blood 200 DSP protocol; EZ1 = EZ1 Advanced XL using EZ1 DSP DNA Blood Kit; QA = QIAamp DNA Blood Mini Kit. The bars show the absolute DNA yield for each sample.

Table 7. Polymorphisms at nucleotide (nt) 667 and nt 1298 of the MTHFR gene detected using the *artus* MTHFR LC PCR Kit

Donor	Method	nt 677	nt 1298	Genotype result
1	QS	Homozygous wt wt677/wt677	Heterozygous variant wt1298/var1298	wt677/wt677 wt1298/var1298 heterozygous variant
	EZ1	Homozygous wt wt677/wt677	Heterozygous variant wt1298/var1298	
	QA	Homozygous wt wt677/wt677	Heterozygous variant wt1298/var1298	
2	QS	Heterozygous variant wt677/var677	Heterozygous variant wt1298/var1298	wt677/var677 wt1298/var1298 heterozygous variant
	EZ1	Heterozygous variant wt677/var677	Heterozygous variant wt1298/var1298	
	QA	Heterozygous variant wt677/var677	Heterozygous variant wt1298/var1298	
3	QS	Homozygous wt wt677/wt677	Heterozygous variant wt1298/var1298	wt677/wt677 wt1298/var1298 heterozygous variant
	EZ1	Homozygous wt wt677/wt677	Heterozygous variant wt1298/var1298	
	QA	Homozygous wt wt677/wt677	Heterozygous variant wt1298/var1298	
4	QS	Homozygous variant var677/var677	Homozygous wt wt1298/wt1298	var677/var677 wt1298/wt1298 homozygous variant
	EZ1	Homozygous variant var677/var677	Homozygous wt wt1298/wt1298	
	QA	Homozygous variant var677/var677	Homozygous wt wt1298/wt1298	
5	QS	Heterozygous variant wt677/var677	Heterozygous variant wt1298/var1298	wt677/var677 wt1298/var1298 heterozygous variant
	EZ1	Heterozygous variant wt677/var677	Heterozygous variant wt1298/var1298	
	QA	Heterozygous variant wt677/var677	Heterozygous variant wt1298/var1298	

Donor	Method	nt 677	nt 1298	Genotype result
6	QS	Heterozygous variant wt677/var677	Heterozygous variant wt1298/var1298	wt677/var677 wt1298/var1298 heterozygous variant
	EZ1	Heterozygous variant wt677/var677	Heterozygous variant wt1298/var1298	
	QA	Heterozygous variant wt677/var677	Heterozygous variant wt1298/var1298	
7	QS	Homozygous wt wt677/wt677	Homozygous wt wt1298/wt1298	wt677/wt677 wt1298/wt1298 homozygous wild-type
	EZ1	Homozygous wt wt677/wt677	Homozygous wt wt1298/wt1298	
	QA	Homozygous wt wt677/wt677	Homozygous wt wt1298/wt1298	
8	QS	Homozygous wt wt677/wt677	Homozygous wt wt1298/wt1298	wt677/wt677 wt1298/wt1298 homozygous wild-type
	EZ1	Homozygous wt wt677/wt677	Homozygous wt wt1298/wt1298	
	QA	Homozygous wt wt677/wt677	Homozygous wt wt1298/wt1298	
9	QS	Heterozygous variant wt677/var677	Heterozygous variant wt1298/var1298	wt677/var677 wt1298/var1298 heterozygous variant
	EZ1	Heterozygous variant wt677/var677	Heterozygous variant wt1298/var1298	
	QA	Heterozygous variant wt677/var677	Heterozygous variant wt1298/var1298	
10	QS	Homozygous wt wt677/wt677	Homozygous wt wt1298/wt1298	wt677/wt677 wt1298/wt1298 homozygous wild-type
	EZ1	Homozygous wt wt677/wt677	Homozygous wt wt1298/wt1298	
	QA	Homozygous wt wt677/wt677	Homozygous wt wt1298/wt1298	

The genetic variance of the methylenetetrahydrofolate reductase (MTHFR) gene was analyzed at two nucleotide positions (nt 677 and nt 1298) through a melting curve analysis on a LightCycler® instrument. Whole blood was collected from 10 healthy donors in BD K2E tubes. For all methods, 200 µl sample input volumes and elution volumes of 200 µl were used.
QS = QIAsymphony DSP DNA Mini Kit and blood 200 DSP protocol; EZ1 = EZ1 Advanced XL using EZ1 DSP DNA Blood Kit; QA = QIAamp DNA Blood Mini Kit; wt = wild-type allele at respective position of MTHFR gene; var = variant allele at respective position of MTHFR gene.

Virus blood

Performance characteristics for virus blood applications were performed using samples from blood donors with a white blood cell count range from 4.0 to 11.0 x 10^6 cells/ml.

Viral DNA recovery

Whole blood was collected from 3 healthy donors in BD K2E tubes and spiked with CMV standard material (titer 3.7 log copies/ml). Viral DNA was purified from 7 replicates, each using the QIAsymphony DSP DNA Mini Kit and the virus blood 200 DSP protocol with 4 different elution volumes (Figure 7).

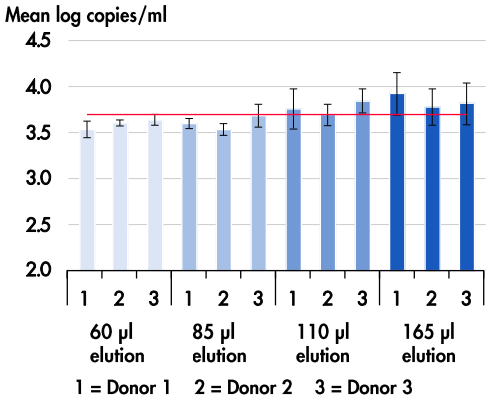

Figure 7. Comparison of viral DNA quantification for different elution volumes. Eluates from each donor sample and elution volume (60 µl, 85 µl, 110 µl, and 165 µl) were analyzed with the *artus* CMV RG PCR Kit. The red line represents the target titer and bars show mean log copies per milliliter with standard deviation.

Inhibitory substances

The influence of inhibitory substances, which may be present in whole blood, on the performance of the virus blood 200 DSP protocol was tested by addition of the following substances: For hemoglobin (200 g/l) and protein (120 g/l), existing levels in the blood sample were determined and additional hemoglobin or protein was added to achieve the indicated concentrations, 200 g/l or 120 g/l, respectively. For bilirubin (200 mg/l) and triglycerides (30 g/l), the total amount of each substance was added to the samples to achieve the indicated concentrations.

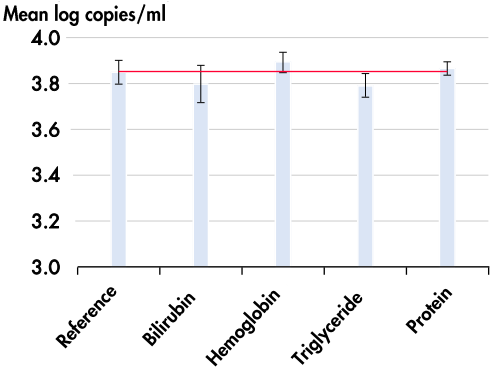

Figure 8. Inhibitory substance test. Whole blood was collected from 1 healthy donor in BD K2E tubes and spiked with CMV standard material (titer 4.0 log copies/ml). Five samples were tested by addition of potential inhibitors and viral DNA was purified from four replicates of each sample using the QIAsymphony DSP DNA Mini Kit and the virus blood 200 DSP protocol with an elution volume of 165 µl. Eluates were analyzed with the *artus* CMV RG PCR Kit. The red line represents the determined titer for reference samples, which were not spiked with any inhibitory substance, and bars show mean log copies per milliliter with standard deviation.

Sensitivity

Hit-rate studies were performed by diluting prequantified CMV WHO standard material in CMV-negative human whole blood. A detection rate of 100% was observed for samples with viral loads of 90 IU of CMV per milliliter (Table 8).

Table 8. Sensitivity of QIAsymphony DSP Virus Blood application

CMV (IU/ml)	Replicates	Hits	Hit %
350	18	18	100.00
230	32	32	100.00
115	31	31	100.00
90	32	32	100.00
60	30	24	80.00
30	30	15	50.00
15	30	10	33.33
6	21	5	23.81
2	21	2	9.52
0	15	0	0.00

Human whole blood was collected from 1 healthy CMV-negative donor in BD K2E tubes and spiked with CMV WHO standard material using different titers. Viral DNA was purified using the QIAsymphony DSP DNA Mini Kit and the virus blood 200 DSP protocol with an elution volume of 60 µl. Eluates were analyzed with the *artus* CMV RG PCR Kit.

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