EZ1® DSP Virus Kit

The EZ1 DSP Virus Kit system performance has been established in performance evaluation studies using plasma, serum, CSF, urine, whole blood, stool, transport media, dried swabs and respiratory samples for isolation of viral nucleic acids and bacterial DNA. Testing was conducted according to the protocols described in the actual Version 4 of the EZ1 DSP Virus Handbook.

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

Serum and plasma

Linear range

The linear range for the EZ1 DSP Virus Kit was evaluated for HCV and HIV-1 RNA viruses and HBV DNA virus. The tests were performed with dilutions of quantified virus panels made in HBV, HCV, and HIV-1 negative human plasma or serum. Dilution series with six different virus titers were tested with 12 replicates each. The linear range of the EZ1 DSP Virus Kit procedure has been determined for HBV, HCV, and HIV-1 with the Abbott RealTime viral load assays (Table 1, Figure 1). RealTime Internal Controls (17 µl each) were added directly to each HIV-1 or HCV sample before extraction. For RealTime HBV, 3.4 µl RealTime HBV Internal Control was combined with carrier RNA for each sample. Viral nucleic acids were extracted from 400 µl samples and eluted in 90 µl elution buffer (AVE). PCR was carried out on the Abbott *m*2000*rt*.

Table 1. Sample source and downstream assays used for determination of linear range of yields with the EZ1 DSP Virus protocol

Virus	Source	Downstream assay	Assay manual used
HIV-1	BBI (Boston Biomedica, Inc., Boston, USA) defective HIV, BBI recalcified plasma	Abbott RealTime HIV-1 (Abbott Molecular Inc.)	Abbott RealTime HIV-1
HCV	ProMedDx (ProMedDx LLC Norton, MA, USA) patient sample, pooled normal human serum	Abbott RealTime HCV (Abbott Molecular Inc.)	Abbott RealTime HCV
HBV	Teragenix (Teragenix Coporate, Ft. Lauderdale, FL, USA) patient sample, recalcified human plasma	Abbott RealTime HBV (Abbott Molecular Inc.)	Abbott RealTime HBV

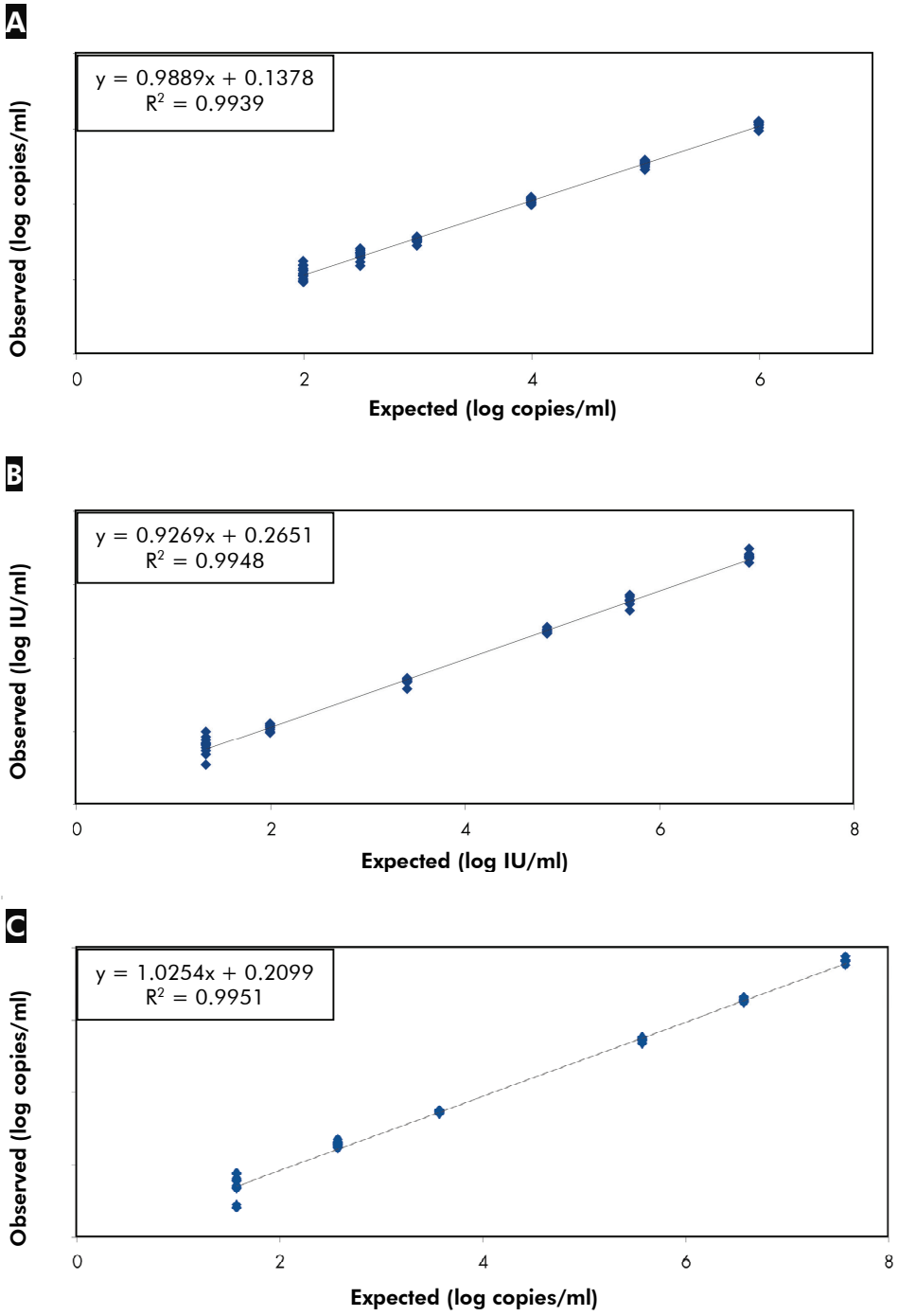

Figure1. Linear range of yields using the EZ1 DSP Virus protocol. The linear range of the EZ1 DSP Virus protocol was determined using viral dilution series and Abbott RealTime assays (Table 1) A for HIV-1, B for HCV, and C for HBV.

Precision

Standard deviations and coefficients of variations (CVs) were determined for HIV-1, HCV, and HBV 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 (Table 1). The inter-assay precision data are shown in Tables 2–4. For each panel member, 12 replicates were extracted in 12 separate runs on the BioRobot EZ1 DSP. PCR was carried out in 2 runs of 6 replicates each on the Abbott *m*2000*rt*.

Table 2. Inter-assay precision of the EZ1 DSP Virus protocol using the Abbott RealTi*me* HIV-1 assay

Panel member	n	Copies/ml	CV (%)	log copies/ml	SD (log copies/ml)
1	12	148	40	2.17	0.17
2	12	426	26	2.63	0.13
3	12	1082	14	3.03	0.06
4	11	11,506	14	4.06	0.06
5	12	116,145	15	5.07	0.07
6	12	1,300,669	16	6.11	0.08

Table 3. Inter-assay precision of the EZ1 DSP Virus protocol using the Abbott RealTi*me* HCV assay

Panel member	n	IU/ml	CV (%)	log IU/ml	SD (log IU/ml)
1	12	39	56	1.59	0.27
2	12	122	22	2.09	0.10
3	12	2331	16	3.37	0.08
4	11	51,582	12	4.71	0.05
5	12	357,547	23	5.55	0.11
6	12	5,505,964	24	6.74	0.10

Table 4. Inter-assay precision of the EZ1 DSP Virus protocol using the Abbott RealTime HBV assay

Panel member	n	Copies/ml	CV (%)	log copies/ml	SD (log copies/ml)
1	12	22	60	1.34	0.34
2	12	357	16	2.55	0.07
3	12	2835	7	3.45	0.03
4	11	280,221	10	5.45	0.05
5	12	3,311,311	12	6.52	0.05
6	12	40,040,547	14	7.60	0.06

Detection limit

The detection limit was determined by the 95% probit value for the EZ1 DSP Virus system using HIV-1 WHO international virus standard 97/656, HBV WHO international virus standard 97/746, and quantified CMV cell-culture supernatant. The detection limit was determined by processing dilution series of the appropriate viruses. The viruses were diluted in HIV-, HBV-, and CMV-negative normal human EDTA plasma pool. Each dilution step was prepared in at least 3 independent runs with at least 6 replicates per dilution. 400 µl plasma was used for sample preparation on the BioRobot EZ1 DSP with elution in 60 µl.

artus® HBV PCR Kits were used for detection of HBV DNA and *artus*® CMV PCR Kits for detection of CMV DNA. The samples were analyzed on a LightCycler® 1.2 Instrument (Roche), a Rotor-Gene® 3000 (Corbett Research), and an ABI PRISM® 7000 SDS (Applied Biosystems). The COBAS® Amplicor® HIV-1Monitor® Test (version 1.5) was used for detection of HIV RNA using the COBAS Amplicor Analyzer. The combined data for all samples were evaluated using probit analysis. The data are presented in Tables 5–6, with representative probit plots in Figures 2–3.

Table 5. Detection limit of HBV and CMV DNA using the EZ1 DSP Virus system and *artus*® PCR Kits

Virus	Input titer	Hits (LightCycler)	Hits (Rotor-Gene)	Hits (ABI PRISM)
HBV	95% probit value (IU/ml)	45.7	14.4	13.2
	Confidence interval (IU/ml)	28–102	9.5–26.5	9.0–23.1
CMV	95% probit value (copies/ml)	67.2	21.8	38.3
	Confidence interval (copies/ml)	41.8–142	14.5–44.1	21.5–89.8

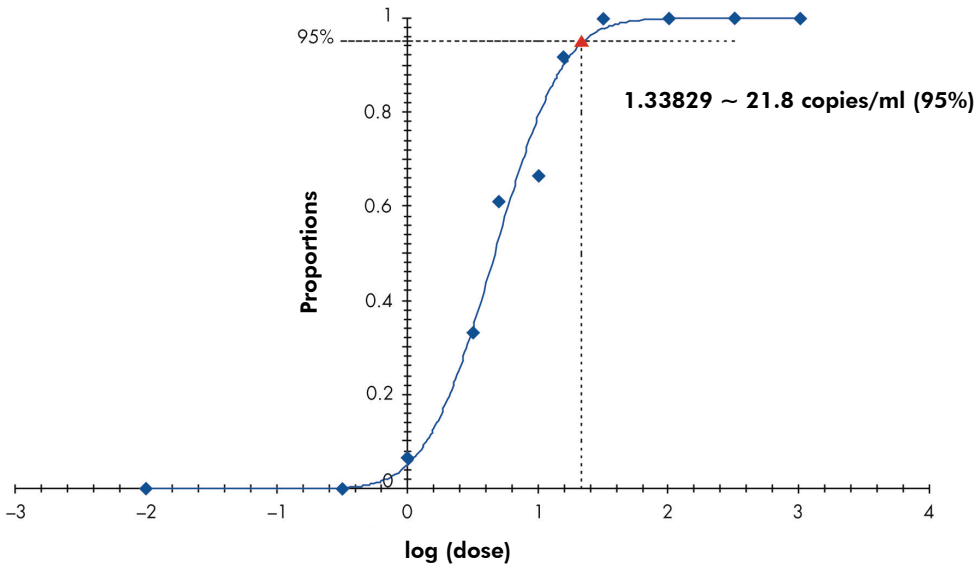

Figure 2. Probit analysis for detection of CMV DNA using the EZ1 DSP Virus system and the *artus*® CMV RG PCR Kit. Viral nucleic acids were purified using the EZ1 DSP Virus system, and the *artus*® CMV PCR RG Kit was used for detection of CMV DNA on the Rotor-Gene 3000. The 95% probit value was 21.8 copies/ml.

Table 6. Detection limit of HIV RNA using the EZ1 DSP Virus system and the COBAS Amplicor HIV-1 Monitor Test, version 1.5

Input titer (IU/ml)	Hits
95% probit value (IU/ml)	114.5
Confidence interval (IU/ml)	82.9–194.3

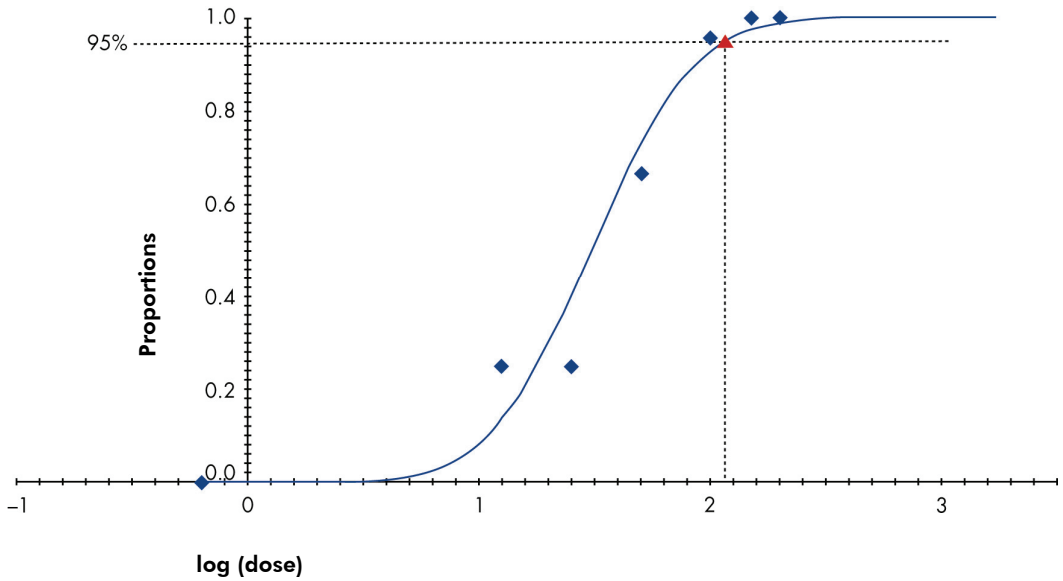

Figure 3. Probit analysis for detection of HIV RNA using the EZ1 DSP Virus system and the COBAS Amplicor HIV-1 Monitor Test, Version 1.5. Viral nucleic acids were purified using the EZ1 DSP Virus system, with 400 µl sample input and 60 µl elution. The COBAS Amplicor HIV-1 Monitor Test was used for detection of HIV RNA on the COBAS Amplicor Analyzer in the ultrasensitive mode. The 95% probit value was 114.5 IU/ml.

Exclusion of sample carryover

Nine runs each on the BioRobot EZ1 DSP, EZ1 Advanced, and EZ1 Advanced XL instruments were performed to evaluate the risk of cross-contamination events during and between EZ1 DSP Virus procedures. The tests were performed using a quantified parvovirus B19 patient sample. The viral load of positive samples used for the carryover tests was 1.0×10^8 IU/ml. For dilution of positive samples and, as negative control samples, a human parvovirus B19 negative EDTA plasma pool was used.

To detect sample-to-sample carryover, 2 runs were performed on each instrument with an alternating checkerboard setup of negative and highly positive samples. Every third run was performed using all negative samples to monitor possible run-to-run carryover. This sample setup was repeated three times resulting in a total of nine runs for each instrument. Parvovirus B19 DNA

was detected and quantitated using the CE-IVD-marked *artus*® Parvo B19 RG PCR Kit on the Rotor-Gene 3000. The analytical detection limit of the *artus*® Parvo B19 RG PCR Kit is determined to be 0.2 IU/µl in the eluate (p = 0.05). This indicates that there is a 95% probability that 0.2 IU/µl in the eluate will be detected.

All of the highly positive samples were detected positive using the *artus*® Parvo B19 RG PCR Kit. All negative samples, in the checkerboard runs and the all-negative runs, were unresponsive (Table 7 shows the results on the BioRobot EZ1 DSP). These experiments demonstrate that the EZ1 DSP Virus protocol provide no sample carryover under these conditions.

Table 7. Cross-contamination test setup and C_T values for detection of parvovirus B19 DNA using the BioRobot EZ1 DSP

	Position					
Run	**1**	**2**	**3**	**4**	**5**	**6**
1	15.47	X	15.41	X	15.36	X
2	X	15.48	X	15.53	X	15.32
3	X	X	X	X	X	X
4	15.35	X	15.2	X	15.27	X
5	X	15.21	X	15.13	X	15.43
6	X	X	X	X	X	X
7	15.62	X	15.48	X	15.23	X
8	X	15.31	X	15.83	X	15.62
9	X	X	X	X	X	X

Mean C_T value of all samples = 15.40 ± 0.18 (CV = 1.14%)

X: Unresponsive after 45 PCR cycles.

Stability

The stability of viral RNA and DNA in eluates generated using the EZ1 DSP Virus Kit was determined. Human EDTA plasma was spiked with $1x10^3$ IU/ml HCV AcroMetrix OptiQuant® HCV RNA and Parvo B19 VQC standard material. Per test time point and incubation condition, 18 replicates were processed using the EZ1 DSP Virus system. Eluates containing Parvo B19 DNA and HCV RNA were incubated for up to 6 hours at 30°C, up to 14 days at 4°C, up to 12 weeks at –20°C, and up to 9 months at –80°C. The study is still ongoing. The eluates were analyzed using a validated in-house HCV RT-PCR and the *artus*® Parvo B19 RG PCR. One RT-PCR failure out of 18 replicates was observed for HCV RNA after storage at 4°C for 14 days (Figure 4).

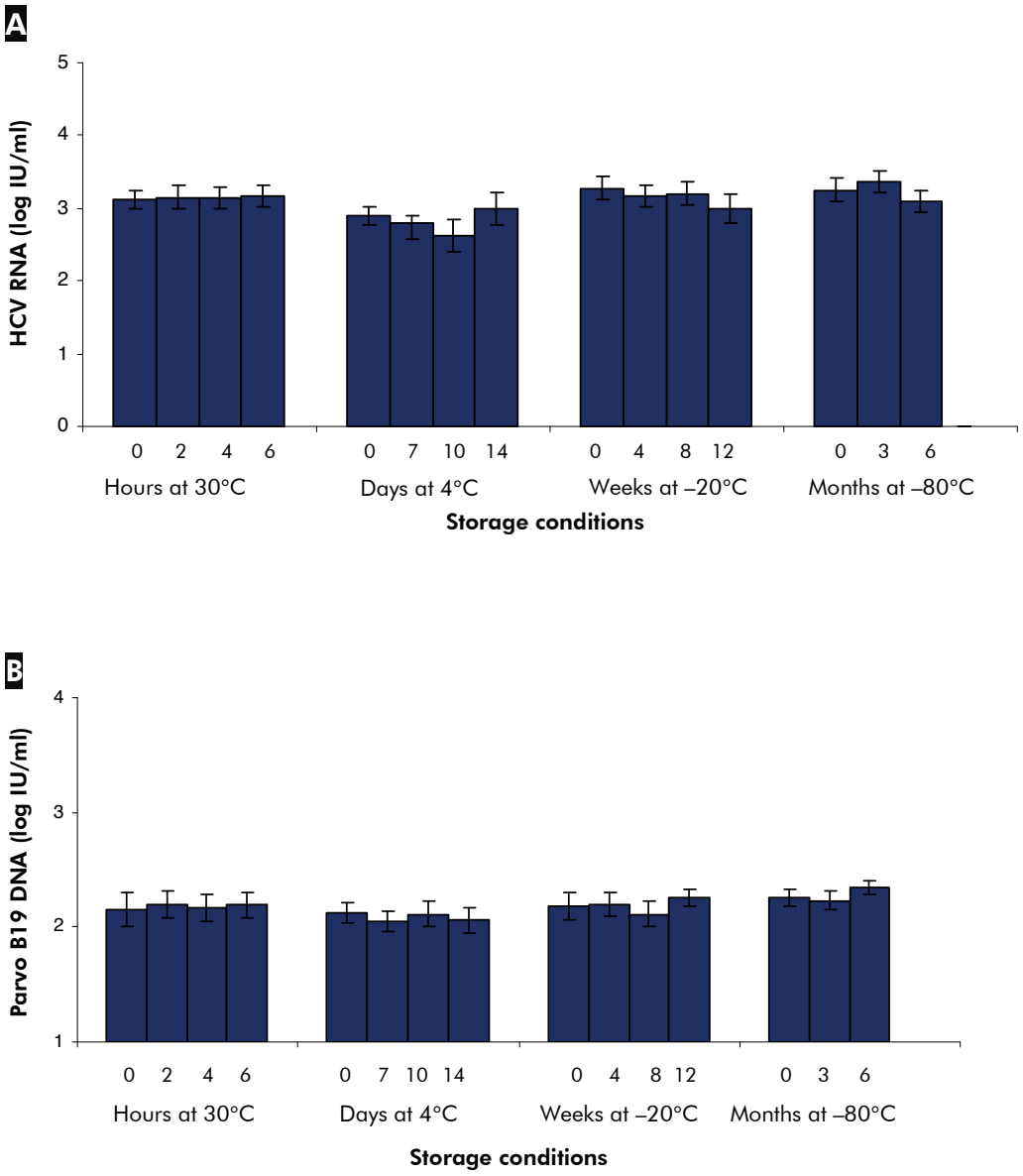

Figure 4. Stability of viral nucleic acids. The stability of viral RNA and DNA in eluates generated using the EZ1 DSP Virus Kit was determined for A HCV RNA and B Parvo B19 DNA.

Reproducibility

The reproducibility was determined using 3 BioRobot EZ1 DSP instruments running on 3 different days (see Table 8, next page). For each test (A–G), 12 replicates were processed in 2 runs on the BioRobot EZ1 DSP. Human EDTA plasma was spiked with $1x10^4$ IU/ml AcroMetrix OptiQuant HCV

RNA and $1x10^3$ IU/ml AcroMetrix OptiQuant HBV DNA. HBV DNA was determined using the *artus*® HBV RG PCR Kit and HCV RNA using a validated in-house HCV RT-PCR assay.

The automated procedure is highly reproducible as demonstrated by comparable results from purification of viral nucleic acids on 3 different BioRobot EZ1 DSP instruments on 3 different days (Figure 5).

Table 8. Reproducibility test setup

Test setup	Day 1	Day 2	Day 3
BioRobot EZ1 DSP I	Test A	Test D	Test F
BioRobot EZ1 DSP II	Test B	Test E	
BioRobot EZ1 DSP III	Test C		Test G

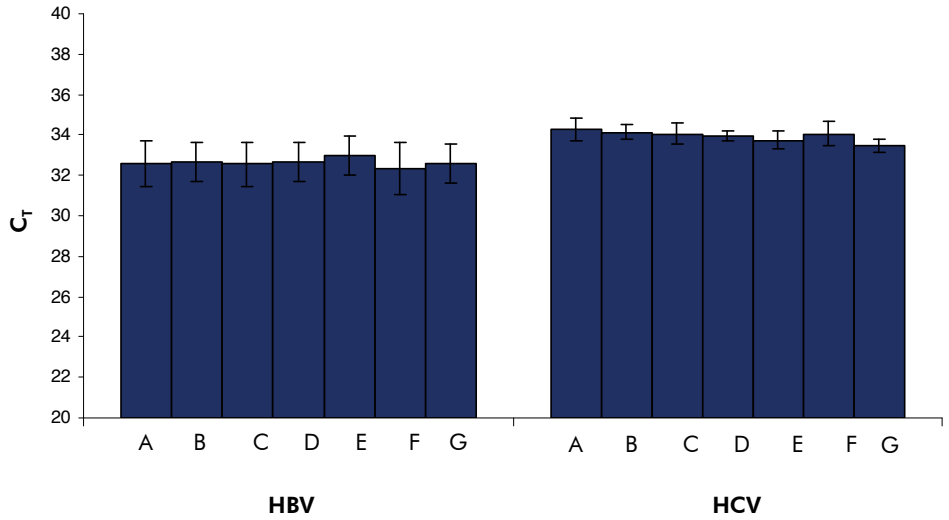

Figure 5. Reproducibility. The reproducibility was determined on three different BioRobot EZ1 DSP instruments on three different days.

Urine

The performance of the EZ1 DSP Virus kit for use with urine samples was evaluated by comparison to plasma using quantified virus panels of CMV (DNA virus) and HCV (RNA virus) diluted in the respective sample material. Urine and plasma samples were treated according to the EZ1 DSP Virus Kit Handbook and equivalent sample volumes were extracted with the EZ1 DSP Virus Kit. Viral nucleic acids were detected using the *artus*® CMV RG PCR and the *artus*® HCV RG RT-PCR Kit. Performance evaluation of the EZ1 DSP Virus Kit comparing urine and plasma showed a discrepancy of only ~2 % (based on C_T values) for both, CMV and HCV (Table 9).

Table 9. Comparison of the EZ1 DSP Virus procedure for use with urine and plasma samples

Specimen type	n	CT value	Ratio Urine/Plasma (CT value)	Copies/ml	Ratio Urine/Plasma (Copies/ml)
			CMV		
Urine	4	31.60	0.98	6,250	1.51
Plasma	5	32.17		4,130	
			HCV		
Urine	4	37.83	1.02	278	0.77
Plasma	5	37.25		363	

Whole Blood

Linear Range

The linear range for the EZ1 DSP Virus Kit was evaluated using EBV as a DNA virus. The tests were performed with dilutions of quantified virus panels made in EBV negative human whole blood. Dilution series with six different virus titers were tested with 4 replicates each. Viral nucleic acids were extracted from 200 µl whole blood (mixed with 200 µl Buffer ATL*) and eluted in 60 µl elution buffer (AVE). The linear range of the EZ1 DSP Virus Kit procedure has been determined for EBV with the *artus*® EBV RG PCR on the Rotor-Gene Q instrument (Figure 6).

*QIAGEN GmbH, cat. no. 939016

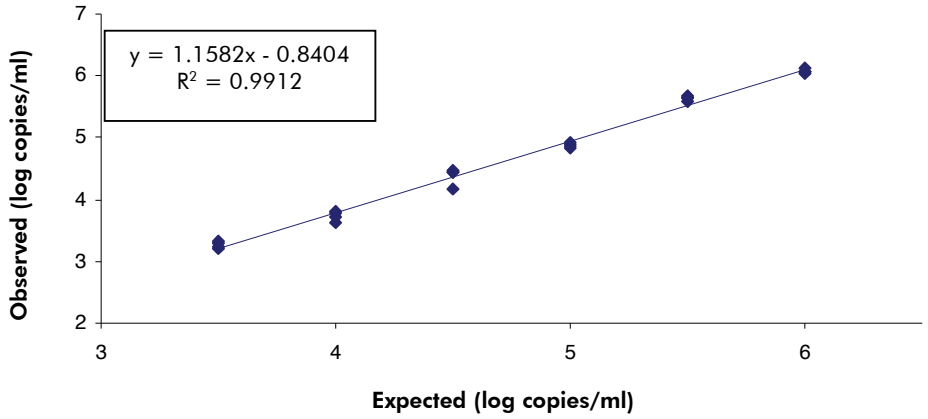

Figure 6. Linear range of yields using the EZ1 DSP Virus protocol in combination with the *artus*® EBV RG PCR assay for the extraction of EBV from whole blood.

Precision

Standard deviations and coefficients of variations (CVs) for whole blood were determined for CMV using the *artus*® CMV RG PCR Kit on the Rotor-Gene Q instrument. The inter-assay precision data are shown in Table 10. Whole blood derived from 13 blood donors was tested in 5 replicates in separate runs on the EZ1 Advanced XL. Viral nucleic acids were extracted from 200 µl whole blood (mixed with 200 µl Buffer ATL*) and eluted in 120 µl elution buffer (AVE).

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Table 10. Inter-assay precision of the EZ1 DSP Virus protocol in combination with the *artus*® CMV RG PCR Kit for extraction of CMV from whole blood

Donor	n	Copies/ml	CV (%)	log copies/ml	SD (log copies/ml)
1	5	7,209	13	3.86	0.06
2	5	7,404	24	3.87	0.10
3	5	7,313	14	3.86	0.06
4	5	7,185	17	3.86	0.08
5	5	7,803	28	3.89	0.12
6	5	7,257	39	3.86	0.17
7	5	7,870	20	3.90	0.08
8	5	7,583	26	3.88	0.12
9	5	8,571	24	3.93	0.10
10	5	7,177	30	3.86	0.13
11	5	8,294	24	3.92	0.11
12	5	7,790	21	3.89	0.10
13	5	7,627	27	3.88	0.13

Stool

Linear Range

The linear range for the EZ1 DSP Virus Kit was evaluated using Adenovirus 5 as a DNA virus. The tests were performed with serial 10-fold dilutions of cell culture supernatant in Adenovirus negative stool. Dilution series with five 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 elution buffer (AVE). The linear range of the EZ1 DSP Virus procedure has been determined in combination with the Adenovirus R-Gene™ PCR assay (Argene SA, France, ref. 96-010B) on the Rotor-Gene Q instrument in comparison to a reference extraction method (Figure 7).
*QIAGEN GmbH, cat. no. 19082

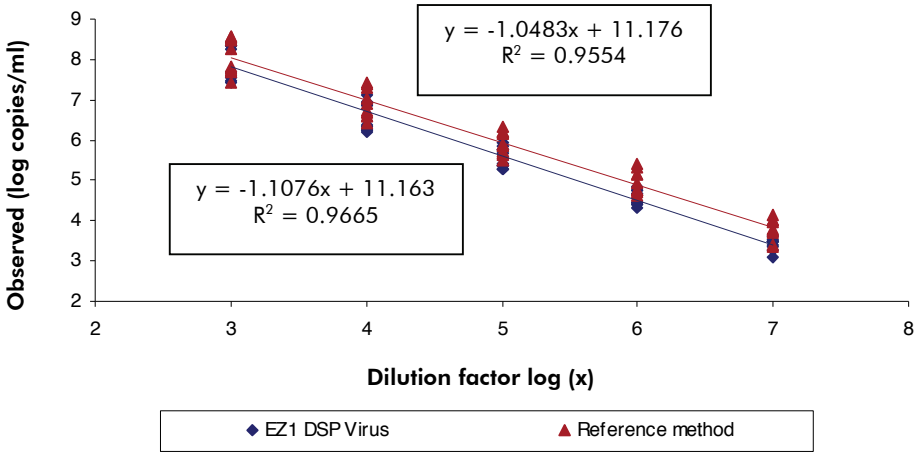

Figure 7. Linear range of yields using the EZ1 DSP Virus protocol in combination with the Adenovirus R-Gene™ PCR assay for the extraction of Adenovirus 5 from stool.

Precision

Standard deviations and coefficients of variations (CVs) for stool were determined for Adenovirus 5 using the Adenovirus R-Gene™ PCR assay (Argene SA, France, ref. 96-010B) on the Rotor-Gene Q instrument. Adenovirus negative stool was spiked with Adenovirus 5 cell culture supernatant and viral DNA was extracted from 200 µl samples (1:10 resuspension in Buffer ASL*) and eluted in 120 µl elution buffer (AVE). Seven EZ1 runs with 9 or 10 replicates each were performed on three days, with three EZ1 Advanced XL instruments and three EZ1 DSP Virus Kit/Buffer ASL lot combinations. All samples were analysed in the same PCR run. The precision data (Table 11) were calculated taking into account results from different instruments, days, lots and all EZ1 runs together (total).
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Table 11. Precision of the EZ1 DSP Virus protocol in combination with the Adenovirus R-Gene™ PCR assay for extraction of Adenovirus 5 from stool

					CV c/ml (%)				
Run	n	Cop/ml	log cop/ml	SD (log cop/ml)	Intra-assay	3 EZ1 Adv. XL	3 days	3 lots	Total
1	9	3,530	3.46	0.22	48	80	59	47	66
2	9	2,955	3.42	0.19	38	–	–	–	–
3	9	2,226	3.26	0.35	43	–	–	–	–
4	9	2,385	3.35	0.23	54	–	–	–	–
5	9	604	2.69	0.24	54	–	–	–	–
6	9	1,214	3.06	0.21	53	–	–	–	–
7	10	1,702	3.19	0.26	48	–	–	–	–

Correlation study

A correlation study was conducted for the EZ1 DSP Virus procedure in comparison to a reference method for the extraction of Norovirus Genogroup II from 66 stool patient samples. Viral nucleic acids were extracted from 200 µl samples (1:10 resuspended in Buffer ASL*) and eluted in 120 µl elution buffer (AVE). Analysis was done with an in-house RT PCR assay against Norovirus Genogroup II (Table 12).

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Table 12. Correlation of the EZ1 DSP Virus procedure with a reference method

		Reference		
		Positive	Negative	Total
EZ1 DSP Virus	Positive	34	15	49
	Negative	1	16	17
	Total	35	31	66

Transport Media

Linear Range

The linear range for the EZ1 DSP Virus Kit was evaluated extracting HSV-1 and *Chlamydia trachomatis (C. trachomatis)* from PreservCyt® medium (Cytyc Corporation, ref. 0200011). The tests were performed with dilutions of quantified virus panels made in transport medium. Dilution series with six different virus titers were tested in 5 or 6 replicates each. The linear range of the EZ1 DSP Virus Kit has been determined in comparison to a reference method with the *artus*® HSV1/2 TM PCR and the *artus*® C. trachomatis TM PCR assay (Figure 8). Viral nucleic acids were extracted from 200 µl samples and eluted in 90 µl elution buffer (AVE).

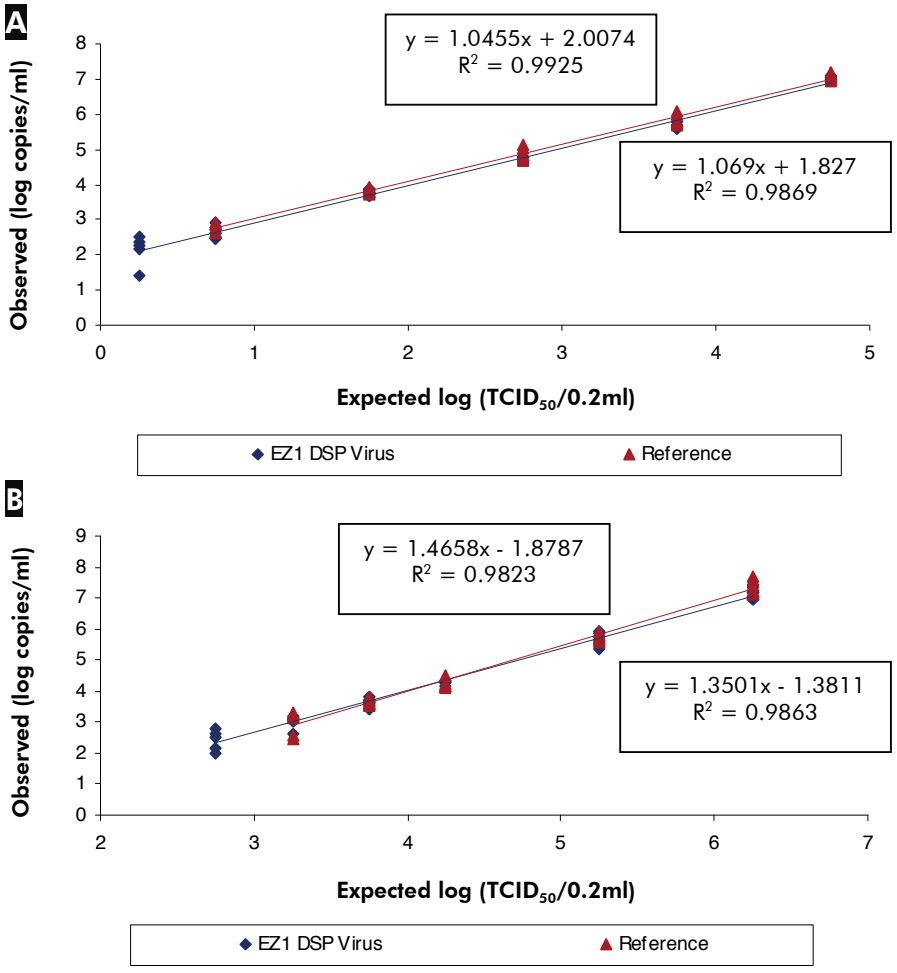

Figure 8. Linear range of yields using the EZ1 DSP Virus protocol in combination with the *artus*® C. trachomatis PCR (A) and the *artus*® HSV1/2 TM TM PCR (B) assay for the extraction of HSV-1 and C. *trachomatis* from transport medium. The study was done in comparison to a reference method.

Precision

Standard deviations and coefficients of variations (CVs) for transport media were determined for HSV-1 and *C. trachomatis* using the *artus*® HSV1/2 TM PCR and the *artus*® C. trachomatis TM PCR assay. Viral and bacterial DNA was extracted from 400 µl medium and eluted in 60 µl elution buffer (AVE). Five transport media were extracted in 12 replicates each in six EZ1runs, on three days and with three EZ1 DSP Virus Kit lots. All samples were analysed in the same PCR run. The intermediate precision for *C. trachomatis* (Table 13) and HSV-1 (Table 14) was calculated taking into account all replicates of each transport medium (different EZ1runs, days and lots).

Table 13. Precision of the EZ1 DSP Virus protocol in combination with the *artus*® C. trachomatis RG PCR Kit for the extraction of *C. trachomatis* from transport media

Medium	n	Nominal log $TCID_{50}$/ 0.2ml	Observed cop/ml	Intermediate precision CV cop/ml (%)	Observed log cop/ml	SD (log cop/ml)
[1]QIAGEN STM	12	3.75	61,623	10	4.79	0.05
[2]Remel M4RT®	12	3.75	79,630	10	4.90	0.05
[3]PreservCyt®	12	3.75	54,749	9	4.74	0.04
[4]BD Surepath®	12	3.75	56,312	18	4.74	0.08
[5]Copan UTM	12	3.75	76,099	9	4.88	0.04

[1] QIAGEN GmbH, cat. no. 5123-1220; [2] Thermo Fisher Scientific Group, ref. R12505; [3] Cytyc Corp., ref. 0200011; [4] Becton, Dickinson and Company, ref. GYN-0001-V; [5] Copan Diagnostics Inc., cat. no. 330C

Table 14. Precision of the EZ1 DSP Virus protocol in combination with the *artus*® HSV1/2 RG PCR Kit for the extraction of HSV-1 from transport media

Medium	n	Nominal log $TCID_{50}$/ 0.2ml	Observed cop/ml	Intermediate precision CV cop/ml (%)	Observed log cop/ml	SD (log cop/ml)
[1]QIAGEN STM	12	4.25	16,615	47	4.17	0.21
[2]Remel M4RT®	12	4.25	17,433	38	4.21	0.20
[3]PreservCyt®	12	4.25	13,494	41	4.09	0.19
[4]BD Surepath®	12	4.25	17,013	58	4.16	0.28
[5]Copan UTM	12	4.25	15,999	39	4.17	0.18

[1] QIAGEN GmbH, cat. no. 5123-1220; [2] Thermo Fisher Scientific Group, ref. R12505; [3] Cytyc Corp., ref. 0200011; [4] Becton, Dickinson and Company, ref. GYN-0001-V; [5] Copan Diagnostics Inc., cat. no. 330C

Clinical Performance (HPV)

Aliquots of DNA purified from a total of 108 samples comprising 50 HC2-positive samples collected in STM, 50 HC2-positive samples collected in PreservCyt® and 8 HC2-negative samples in STM were tested with the *digene*® HPV Genotyping RH Test (cat. no. 613413) and the *digene*® HPV Genotyping LQ Test (cat. no. 613215) in comparison to the Free University RLB system*.

Results were scored as either identical (100% matching genotypes), compatible (at least one genotype in common), or discordant (no matching genotypes). Discrepancies (discordant genotyping results) were resolved by repeating both assays and, in case of remaining discrepancies, by subsequent analysis with a third sensitive HPV detection and genotyping assay [SPF10-LiPA25 (version1)].

The results showed a very low level of discrepant samples (2%) after resolution of initial discrepant samples for both genotyping assays compared to the reference method (Table 15.)

Table 15. Comparison of the digene HPV Genotyping RH Test (A) and the digene HPV Genotyping LQ Test with the Free University RLB system* using the EZ1 DSP Virus procedure for extraction of HPV from transport medium

	A	B
Result type	**% of clinical samples**	**% of clinical samples**
Identical	80	58
Compatible	18	12
Discrepant	2	2

* van den Brule, A. J., Pol R., Fransen-Daalmeije, N., Schouls, L. M., Meijer, C. J., and Snijders, P. J. (2002) GP5+/6+ PCR followed by reverse line blot analysis enables rapid and high-throughput identification of human papillomavirus genotypes. J Clin Microbiol 40, 779.

Clinical Performance (Influenza A)

To demonstrate clinical performance, 102 characterized nasopharyngeal swab specimens collected in UTM (Copan Diagnostics Inc., cat. no. 330C) were evaluated using the EZ1 DSP Virus Kit for nucleic acid extraction. Influenza A RNA was detected using the *artus*® Inf. A H1N1 2009 LC RT-PCR Kit and the EUA approved Focus Influenza A H1N1 (2009) Real-Time RT-PCR test (Table 16).

Table 16. Comparison of the *artus*® Inf. A H1N1 2009 LC RT-PCR Kit against the EUA approved Focus Influenza A H1N1 (2009) Real-Time RT-PCR test using the EZ1 DSP Virus Kit for extraction of seasonal Influenza A and 2009 H1N1 Influenza virus from nasopharyngeal swabs

		Focus Influenza A H1N1 (2009) Real-Time RT-PCR			
		Seasonal Infl.A positive	**2009 H1N1 positive**	**Negative**	**Total**
***artus*® Inf. A H1N1 2009 LC RT-PCR**	**Seasonal Infl.A positive**	5	0	2	7
	2009 H1N1 positive	0	27	1	28
	Negative	0	0	67	67
	Total	5	27	70	102

Dried Swabs

Linear Range

The linear range for the EZ1 DSP Virus Kit was evaluated extracting HSV-1 and *Chlamydia trachomatis (C. trachomatis)* from Puritan Cotton Swabs (ref. 25-806 1PC, Puritan Medical Products Co. LLC). The tests were performed with dilutions of quantified standard material. Human negative saliva was spiked with pathogen material and transferred to the swab. After dehydration, pathogens were reisolated from the dried swab by resuspension in 600 µl Buffer ATL*. Dilution series with six different virus titers were tested in 5 or 6 replicates each. The linear range of the EZ1 DSP Virus Kit has been determined in comparison to a reference method with the *artus*® HSV1/2 TM PCR and the *artus*® C. trachomatis TM PCR assay (Figure 9). Viral nucleic acids were extracted from 400 µl samples and eluted in 150 µl elution buffer (AVE).

*QIAGEN GmbH, cat. no. 939016

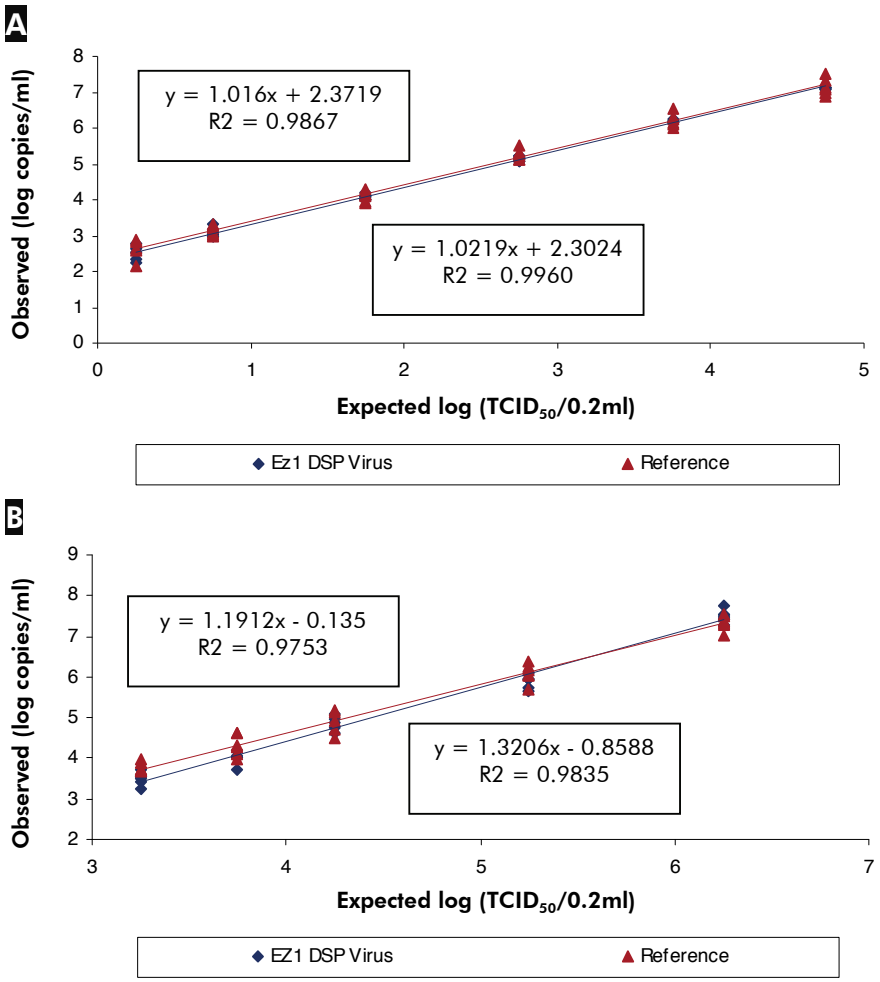

Figure 9. Linear range of yields using the EZ1 DSP Virus protocol in combination with the *artus*® C. trachomatis PCR (A) and the *artus*® HSV1/2 TM TM PCR (B) assay for the extraction of C. trachomatis and HSV-1 from dried cotton swabs. The study was done in comparison to a reference method.

Precision

Standard deviations and coefficients of variations (CVs) for dried swabs were determined for HSV-1 and C. *trachomatis* using the *artus*® HSV1/2 TM PCR and the *artus*® C. trachomatis TM PCR assay. Copan Flocked Swabs (cat. no. 502CS0, Copan Italia S.p.A.) and Puritan Cotton Swabs (ref. 25-806 1PC, Puritan Medical Products Co. LLC) Dried swabs were prepared and pretreated as described above and viral and bacterial DNA was extracted from 400 µl sample volume and eluted in 60 µl elution buffer (AVE). Extraction was done with three saliva donors in 8 or 9 replicates each, in six EZ1runs, on three days and with three EZ1 DSP Virus Kit/Buffer ATL lot combinations. All

samples were analysed in the same PCR run. The intermediate precision for C. *trachomatis* (Table 17) and HSV-1 (Table 18) was calculated taking into account all replicates of each donor and swab type (different EZ1runs, days and lots).

Table 17. Precision of the EZ1 DSP Virus protocol in combination with the *artus*® C. trachomatis RG PCR Kit for the extraction of C. *trachomatis* from dried swabs

Swab type	Donor	n	Nominal log $TCID_{50}$/ 0.2 ml	Observed cop/ml	Intermediate precision CV cop/ml (%)	Observed log cop/ml	SD (log copies/ ml)
Puritan cotton swabs	1	9	1.75	16,782	28	4.22	0.12
	2	9	1.75	15,896	23	4.20	0.09
	3	9	1.75	16,111	12	4.21	0.05
Copan flocked swabs	1	9	1.75	26,486	19	4.42	0.09
	2	9	1.75	30,356	17	4.48	0.08
	3	9	1.75	19,926	18	4.30	0.08

Table 18. Precision of the EZ1 DSP Virus protocol in combination with the *artus*® HSV1/2 RG PCR Kit for the extraction of HSV-1 from dried swabs

Swab type	Donor	n	Nominal log $TCID_{50}$/ 0.2 ml	Observed cop/ml	Intermediate precision CV cop/ml (%)	Observed log cop/ml	SD (log copies/ ml)
Puritan cotton swabs	1	9	3.75	5,843	52	3.77	0.22
	2	8	3.75	13,295	62	4.12	0.20
	3	8	3.75	10,272	40	4.01	0.16
Copan flocked swabs	1	8	3.75	6,215	30	3.79	0.13
	2	9	3.75	10,773	24	4.03	0.11
	3	9	3.75	10,336	24	4.01	0.11

Respiratory samples (sputum)

Correlation study

A correlation study was conducted for the EZ1 DSP Virus for the extraction of *Mycobacterium tuberculosis* from negative human sputum. A dilution series with 4 different virus titers was tested in single replicates in comparison to a reference method. Bacterial DNA was extracted from 200 µl sputum, pretreated with Sputasol (Oxoid Limited, ref. SR0233) and lysozyme (Sigma-Aldrich, cat. no. L6876) as described in the EZ1 DSP Virus Handbook Version 4, and eluted in 90 µl elution buffer (AVE). Analysis was done with the *artus*® M. tuberculosis RG PCR assay (Figure 10).

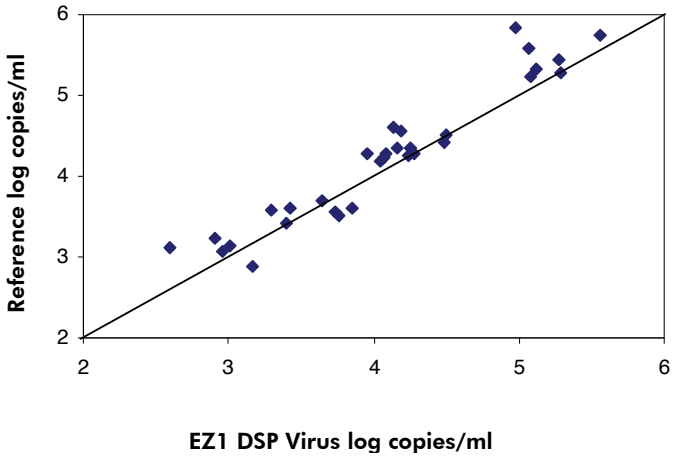

Figure 10. Correlation of the EZ1 DSP Virus procedure with a reference method.

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