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Performance evaluation of the QIAGEN EZ1 DSP Virus Kit with Abbott RealTime HIV-1, HBV and HCV assays

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ABSTRACT

Background: Automated sample preparation systems must meet the demands of routine diagnostics laboratories with regard to performance characteristics and compatibility with downstream assays.
Objectives: In this study, the performance of QIAGEN's EZ1 DSP Virus Kit on the BioRobot EZ1 DSP was evaluated in combination with the Abbott RealTime HIV-1, HCV, and HBV assays, followed by thermalcycling and detection on the Abbott *m2000rt* platform.
Study design: The following performance characteristics were evaluated: linear range and precision, sensitivity, cross-contamination, effects of interfering substances and correlation.
Results: Linearity was observed within the tested ranges (for HIV-1: 2–6 log copies/ml, HCV: 1.3–6.9 log IU/ml, HBV: 1.6–7.6 log copies/ml). Excellent precision was obtained (inter-assay standard deviation for HIV-1: 0.06–0.17 log copies/ml (>2.17 log copies/ml), HCV: 0.05–0.11 log IU/ml (>2.09 log IU/ml), HBV: 0.03–0.07 log copies/ml (>2.55 log copies/ml)), with good sensitivity (95% hit rates for HIV-1: 50 copies/ml, HCV: 12.5 IU/ml, HBV: 10 IU/ml). No cross-contamination was observed, as well as no negative impact of elevated levels of various interfering substances. In addition, HCV and HBV viral load measurements after BioRobot EZ1 DSP extraction correlated well with those obtained after Abbott *m2000sp* extraction.
Conclusions: This evaluation demonstrates that the QIAGEN EZ1 DSP Virus Kit provides an attractive solution for fully automated, low throughput sample preparation for use with the Abbott RealTime HIV-1, HCV, and HBV assays.

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1. Background

Hepatitis B and C and human immunodeficiency virus type 1 cause significant morbidity and mortality worldwide. A sensitive and accurate quantification of the respective viral RNA or DNA level is crucial for the identification of patients who need to be treated, for the decision about treatment duration, and for monitoring the virologic response and resistance to antiviral treatment.[1–4] Further, such quantitative data can be informative in establishing a prognosis of e.g. chronic HBV-related liver disease or hepatocellular carcinoma.[5–7]

Automated solutions for use in purification of viral nucleic acids and their quantitative detection minimize the risk of user-to-user variability and human error, standardize the process, offer more safety in handling infectious starting materials, and reduce hands-on time.

The EZ1 DSP Virus Kit provides a generic, fully automated system utilizing magnetic silica-bead technology on the QIAGEN BioRobot EZ1 DSP instrument. It enables simultaneous purification and isolation of viral DNA and RNA from up to six human plasma, serum or CSF samples. The purification procedure comprises lysis, binding, washing and elution, and is designed to ensure safe and reproducible handling of potentially infectious samples. In this study, the performance of the QIAGEN EZ1 DSP Virus Kit was evaluated in combination with the Abbott RealTime HIV-1, HCV, and HBV assays. The Abbott RealTime assays are quantitative *in vitro* polymerase chain reaction assays which utilize fluorescently labeled oligonucleotide probes to detect specific amplified product in a homogenous realtime assay format. The assays include external run controls as well as an internal control which is amplified non-competitively to monitor sample inhibition and recovery.

Abbreviations: CV, coefficient of variation; IC, internal control; S.D., standard deviation.

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2. Objective

The aim of this work was to evaluate the efficiency of automated nucleic acid isolation from human plasma and serum samples using the EZ1 DSP Virus kit for use with the Abbott RealTi*me* HBV, HCV, and HIV-1 assays. Also, performance of the EZ1 DSP kit and the BioRobot EZ1 DSP was compared to that of the Abbott *m*Sample Preparation System kits and the *m*2000*sp* instrument.

3. Study design

3.1. Sample material

Panels were prepared by diluting the high titer viral stocks (defective HIV-1 virions from Boston Biomedica, Inc., HCV specimen from ProMedDx LLC, and HBV specimen from Teragenix Corporation) in negative human plasma (BBI) or pooled normal human serum (ProMedDx) (HCV only). For the sensitivity studies, the WHO International Standard for HBV DNA (97/746), the VQA HIV-1 virion stock and the 2nd WHO International Standard for HCV RNA (96/798) were diluted in negative plasma to the levels indicated in Table 3. Fifty-nine HCV- or HBV-positive clinical specimens were used for correlation studies comparing the BioRobot EZ1 DSP and the *m*2000*sp* sample preparation methods.

3.2. Nucleic acid isolation

Viral nucleic acids were extracted from 0.4 ml specimens on the QIAGEN BioRobot EZ1 DSP instrument using the EZ1 DSP Virus Kit (List No. D053696) as described in the package inserts (QIAGEN GmbH, Hilden, Germany). For this study an elution volume of 90 μl was selected. To incorporate the internal controls into the sample extraction, 17 μl of RealTi*me* HIV-1 or HCV internal control was added directly to each HIV-1 or HCV sample before extraction. For each HBV sample, a premix of 3.4 μl internal control, 3.6 μl of cRNA and 53 μl of AVE were placed into the respective IC slot. Sample extraction on the Abbott *m*2000*sp* was carried out according to the package inserts (Abbott Molecular, Inc., Des Plaines, IL, List Nos. 2G31, 4J86 and 2G34).

3.3. Realtime PCR

PCR mastermix and reaction assembly were performed as described in the assay package inserts. PCR amplification and real time detection were carried out on the Abbott *m*2000*rt*. Assay run controls were extracted using the BioRobot EZ1 DSP and one set of run controls was included on each PCR plate. Assay calibrators were extracted using the BioRobot EZ1 DSP and sample quantification was performed either from calibrators on the same PCR run or from a stored calibration curve.

3.4. Interfering substances

The effect of elevated levels of endogenous substances on the quality of the EZ1 DSP Virus Kit sample eluates was evaluated. Potentially interfering substances were added to decalcified negative plasma to the following final concentrations: (i) hemoglobin 0.5 g/dl (Sigma); (ii) bilirubin 0.02 g/dl (Sigma); (iii) triglycerides 3 g/dl (Abbott); (iv) protein 9 g/dl (Sigma). HIV-1, HBV (1000 copies/ml), and HCV (1000 IU/ml) were added to panels containing the potentially interfering substances.

3.5. Cross-contamination

The potential for well-to-well cross-contamination on the BioRobot EZ1 DSP was evaluated in six runs alternating high posi-

Table 1
Samples with a high viral load (∼9 log copies/ml HBV) (+) and HBV-negative samples (−) were placed in the instrument in an alternating order to analyze the EZ1 DSP virus protocol with respect to potential cross-contamination. The order of positive and negative samples was changed from run to run.

Run	Well 1	Well 2	Well 3	Well 4	Well 5	Well 6
1	–	+	–	+	–	+
2	+	–	+	–	+	–
3	–	+	–	+	–	+
4	+	–	+	–	+	–
5	–	+	–	+	–	+
6	+	–	+	–	+	–

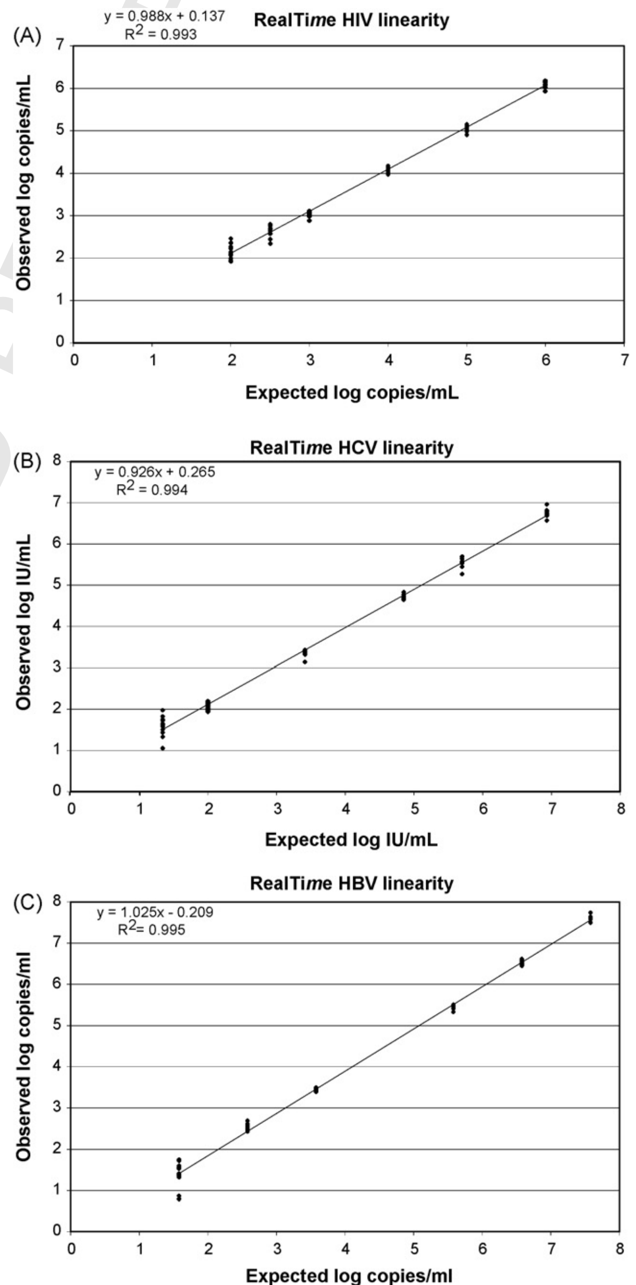

Fig. 1. Linearity of viral load determination using the EZ1 DSP Virus kit in combination with the *m*2000*rt* assay targeting HIV-1 RNA (A), HCV RNA (B), and HBV DNA (C). Expected versus observed concentration are shown on the *x*- and *y*-axes, respectively, in log copies/ml or log IU/ml. For every parameter, 12 replicates of 6 different dilutions were analyzed.

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Table 2
Precision of viral load determination using the EZ1 DSP Virus Kit in combination with the *m2000rt* assay targeting HIV-1 RNA, HCV RNA, and HBV DNA.

HIV-1	Panel member	No. of replicates	Mean (copies/ml)	Inter-assay CV (%)	Mean (log copies/ml)	Inter-assay S.D. (log copies/ml)
	1	12	148	40	2.17	0.17
	2	12	426	26	2.63	0.13
	3	12	1,082	14	3.03	0.06
	4	12	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

HCV	Panel member	No. of replicates	Mean (IU/ml)	Inter-assay CV (%)	Mean (log IU/ml)	Inter-assay S.D. (log IU/ml)
	1	12	39	56	1.59	0.27
	2	12	122	22	2.09	0.10
	3	12	2,331	16	3.37	0.08
	4	12	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

HBV	Panel member	No. of replicates	Mean (copies/ml)	Inter-assay CV (%)	Mean (log copies/ml)	Inter-assay S.D. (log copies/ml)
	1	12	22	60	1.34	0.34
	2	12	357	16	2.55	0.07
	3	12	2,835	7	3.45	0.03
	4	12	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

CV: coefficient of variation and S.D.: standard deviation.

tive HBV sample (~9 log copies/ml) with negative plasma as shown in Table 1.

4. Results

4.1. Linear range

The linear range of the HIV-1, HCV and HBV assays was evaluated, with 12 replicates per virus level. For HIV-1, a panel ranging from 2 to 6 log copies/ml was analyzed. Within this range, a good correlation was found between the average HIV-1 RNA levels measured and the expected levels ($R^2 = 0.994$) (Fig. 1A). HCV RNA and HBV DNA panels ranged from 1.3 to 6.9 log IU/ml for HCV and 1.6–7.6 log copies/ml for HBV (Fig. 1B and C). Again, R^2 values of 0.995 displayed a high concordance between measured and expected nucleic acid levels for the viral load range analyzed.

4.2. Precision

The same panels that were used for determination of the linear range were also used to evaluate precision. Standard deviations (S.D.s) and coefficients of variations (CVs) were determined for HIV-1, HCV, and HBV dilution series in the linear range of the assays. Inter-assay S.D.s represent the accumulation of intra-run and between-run variation (Table 2).

The data reveal that the inter-assay standard deviation is small throughout the HIV-1 RNA quantitation range analyzed, with values between 0.06 and 0.17 log copies/ml (Table 2). Excellent precision was also observed across a broad range of HCV RNA levels: S.D. values ranged between 0.05 and 0.11 log IU/ml for most HCV levels, a small increase in S.D. (0.27 log IU/ml) was found at an HCV RNA level of 39 IU/ml (Table 2). Similarly, S.D. values between 0.03 and 0.07 log copies/ml were observed for most HBV DNA levels, whereas a higher S.D. value of 0.34 log copies/ml was obtained for samples with a viral load of 22 copies/ml (Table 2).

4.3. Sensitivity

To investigate the sensitivity of the whole system consisting of the EZ1 DSP Virus Kit sample preparation and the *m2000rt* PCR assays, three different hit rate studies were performed. A VQA and two WHO International Standards were diluted in negative plasma to the levels indicated in Table 3. Twenty replicates of each dilution were tested. 100% of the HIV-1 RNA samples with a viral load of 100 copies/ml and 95% of the samples at 50 copies/ml were detected (Table 3). All 20 replicates with a titer of 25 IU/ml HCV RNA and 18 of the 19 replicates with a titer of 12.5 IU/ml were successfully detected (Table 3). Finally, the system was challenged with respect to the detection of viral DNA. As shown in Table 3, 100% of samples at 20 IU/ml of HBV and 95% of samples at 10 IU/ml were detected.

4.4. Correlation between the BioRobot EZ1 DSP and the m2000sp sample preparation methods

Results for 29 HCV-positive and 30 HBV-positive specimens extracted on the BioRobot EZ1 DSP were compared to results determined after extraction on the Abbott *m2000sp*. Results are depicted in Fig. 2. The R^2-value was 0.943 for HCV (Fig. 2A) and 0.986 for

Table 3
Sensitivity of viral load determination using the EZ1 DSP Virus Kit in combination with the different *m2000rt* assays.

HIV-1			HCV			HBV		
Conc. (copies/ml)	No. of pos./no. of tested samples	Hit rate (%)	Conc. (IU/ml)	No. of pos./no. of tested samples	Hit rate (%)	Conc. (copies/ml)	No. of pos./no. of tested samples	Hit rate (%)
100.0	20/20	100	25.0	20/20	100	20.0	20/20	100
60.0	19/20	95	15.0	18/20	90	10.0	19/20	95
50.0	19/20	95	12.5	18/19	95	5.0	16/20	80
30.0	18/20	90	10.0	17/20	85	2.5	12/20	60
10.0	11/20	55	7.5	16/20	80	1.0	4/20	20
5.0	7/20	35	5.0	13/20	65	0.5	6/20	30

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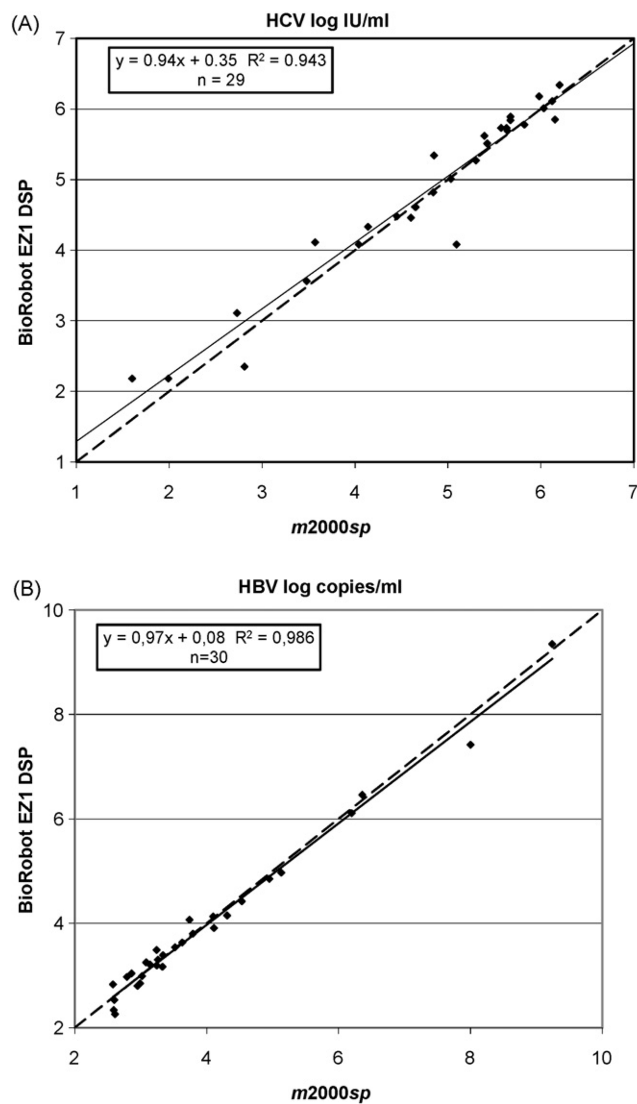

Fig. 2. Correlation for HCV RNA (A) and HBV DNA (B) determinations of 59 clinical samples within the dynamic range of the respective *m2000rt* assay using the *m2000sp* and the EZ1 DSP Virus sample preparation. The dashed line represents the identity line and the solid line indicates the obtained regression line.

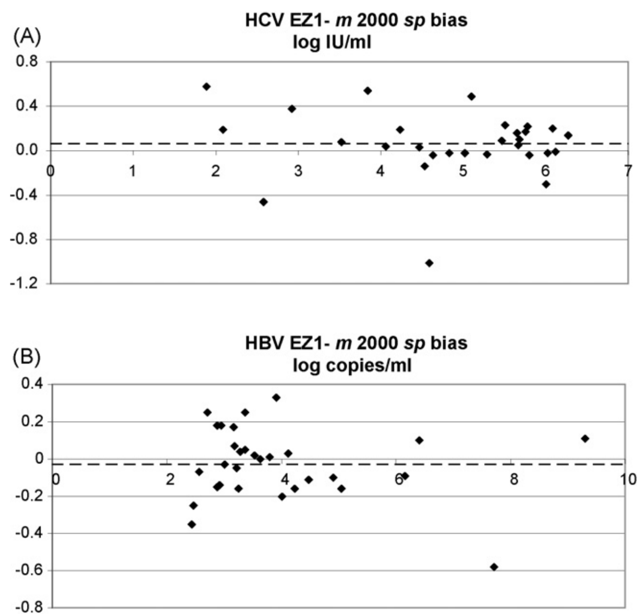

Fig. 3. Agreement plots between the *m2000sp* and the BR EZ1 DSP sample preparations in combination with the *m2000rt* HCV assay (A) and the *m2000rt* HBV assay (B). The average concentration (*x*-axis) is plotted versus the difference (*y*-axis). The dashed line represents the mean difference for the samples.

HBV (Fig. 2B), showing a good agreement for the two different sample preparation methods. The mean EZ1–*m2000sp* bias was 0.06 log IU/ml for HCV and −0.03 copies/ml for HBV, as revealed by Bland–Altman analysis (Fig. 3A and B).

4.5. Influence of potentially inhibiting substances

Lack of purification efficiency can lead to carry over of substances inhibitory to PCR thereby negatively impacting the exact determination of viral load in clinical samples. Therefore, the effect of elevated levels of endogenous substances on the quality of the EZ1 DSP Virus eluates was evaluated. Four potentially interfering substances were spiked into decalcified negative plasma with concentrations significantly greater than their physiological ranges in healthy individuals.

Viral particles were added to panels containing the potentially interfering substances targeting 1000 copies/ml of HIV-1 and HBV and 1000 IU/ml of HCV. No significant effect on the performance of the Abbott RealT*ime* assays were observed in the presence of the potentially interfering substances for all samples tested (Table 4). The largest interfering effect was measured with a difference of 0.20 log copies/ml and 0.34 log IU/ml for HIV and HCV, respectively, after the addition of 0.5 g/dl hemoglobin.

4.6. Cross-contamination

The potential for well-to-well cross-contamination on the BioRobot EZ1 DSP was evaluated in six runs alternating high positive HBV panel (∼9 log copies/ml) with negative plasma as shown in Table 1. Positive samples were detected with an average Ct of 13.43 (data not shown), reflecting the high viral load of these samples. No HBV DNA was detected in the eighteen negative plasma sample eluates despite their spatial proximity to the positive samples.

5. Discussion

Closed-tube real time detection methods of PCR or NASBA amplicons allow sensitive detection of viral nucleic acids while minimizing the risk of contamination which may cause misquantification or false positive results. Further, they have the advantage of a broad linear range. Abbott has developed quantitative viral load tests for HIV-1, HCV and HBV utilizing real time PCR. It has already been shown that the combination of the *m2000sp* sample preparation procedures (either DNA or RNA) and the respective *m2000rt* assay are suitable to monitor viral loads in plasma or serum.[8–11] The aim of this study was to evaluate the *m2000rt* assays in combination with an alternative low- to medium-throughput sample preparation procedure, QIAGENs EZ1 DSP Virus kit used on the BioRobot EZ1 DSP. Similar to the *m2000sp* kits, the EZ1 DSP Virus kit uses magnetic bead technology to purify viral nucleic acids. One benefit of the EZ1 DSP Virus kit is the capability to co-purify both viral RNA and DNA with one chemistry and one purification protocol.

This study has shown that EZ1 DSP Virus kit eluates are suitable for a subsequent analysis with the Abbott PCR assays: (i) no cross-contamination has been detected using samples at a concentration of ∼9 log copies/ml. This confirms the suitability of the system to analyze samples with high viral loads without producing falsely positive results. (ii) The measured high purification effi-

Table 4
Analysis of the effect of potentially interfering substances. HIV-1 virions, HCV virions, or HBV virions were added to plasma containing one of four different potentially interfering substances being present in the denoted concentrations. The mean viral load of 5 replicates was determined and compared to the determined mean viral load of the control samples without additional substances.

	Condition	Control	Bilirubin 0.02 g/dl	Hemoglobin 0.5 g/dl	Protein 9.0 g/dl	Triglycerides 3.0 g/dl
HIV-1	Mean (log copies/ml)	3.10	3.10	2.90	2.99	2.97
	S.D.	0.05	0.06	0.08	0.05	0.03
	Condition–control (log copies/ml)		0.00	−0.20	−0.11	−0.13
HCV	Mean (log IU/ml)	3.09	3.11	2.75	3.00	2.92
	S.D.	0.08	0.07	0.19	0.19	0.14
	Condition–control (log IU/ml)		0.02	−0.34	−0.09	−0.17
HBV	Mean (log copies/ml)	2.87	2.83	2.80	2.77	2.80
	S.D.	0.04	0.02	0.09	0.06	0.06
	Condition–control (log copies/ml)		−0.04	−0.07	−0.10	−0.07

208 ciency ensured that even excess amounts of various potentially 209 interfering substances in the samples did not interfere. Even with 210 a hemoglobin concentration exceeding the normal physiological 211 range by a factor of about 100, a difference of only 0.2 log copies/ml 212 and 0.34 log IU/ml for HIV and HCV, respectively, was observed.

213 With respect to the three tested viral DNA and RNA assays, the 214 EZ1 DSP Virus kit enables very efficient isolation for samples over a 215 broad dynamic range: (i) eluates derived from the EZ1 DSP sam- 216 ple preparation gave linear results within the dynamic range of 217 the respective PCR assays: for HIV-1, samples covering the range 218 of 2–6 log copies/ml were analyzed, whereas the analyzed dynamic 219 range for HBV samples covered greater than 6 log, from 1.3 to 220 7.6 log copies/ml. (ii) At the lower ends of each dynamic range sam- 221 ples with low viral loads were successfully detected: 100% of HIV-1 222 samples with 100 copies/ml, of HCV samples with 25 IU/ml, and of 223 HBV samples with 20 IU/ml were detected.

224 High precision, as well as high sensitivity is crucial to a system 225 of nucleic acid purification and subsequent detection. The RealT*im*e 226 assays tested displayed inter assay S.D.s generally less than 0.10 log 227 and no higher than 0.13 log except at the lowest level tested in each 228 assay. The combination of the EZ1 DSP Virus Kit and these Abbott 229 RealT*im*e assay results in highly reproducible results.

230 Taken together, these data suggest that the EZ1 DSP Virus kit 231 with the respective protocol is a suitable low throughput alterna- 232 tive to the *m*2000*sp* extraction procedure. To test the extraction 233 capabilities of the EZ1 with clinical samples, both sample prepara- 234 tion systems were directly compared with respect to quantification 235 with the *m*2000*rt* HCV and HBV assays. Clinical samples (plasma 236 and serum) with viral loads spanning the dynamic range of the 237 downstream assays were used for the analysis. For both assays, the 238 relative agreement between the determined viral loads was good, 239 with only minor mean biases. Looking at the individual results, 56 of 240 the 59 samples show a viral load difference of less than 0.5 log IU/ml. 241 For HBV both the prediction of the early virologic response and 242 the detection of drug-resistant strains are based on viral load dif- 243 ferences of 1 log[12]; thus, a difference below 0.5 log IU/ml among 244 different quantitative methods is clinically acceptable.

245 Overall, the QIAGEN EZ1 DSP Virus Kit enables reproducible 246 purification of viral nucleic acids from human plasma or serum and 247 allows for reliable, sensitive, and specific detection and quantifica- 248 tion of RNA and DNA viruses by the Abbott RealT*im*e HIV-1, HCV, and HBV assays.

Conflict of interest Q1 249

250 There is no conflict of interest.

Acknowledgement 251

252 None.

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