Nucleic Acid Extraction from 400 µL sample volume using MagDEA Dx SV

(Application example of MagDEA Dx SV, a nucleic acid extraction reagent for the fully automated magLEAD systems)

Precision System Science Co., Ltd.

〈Introduction〉

Precision System Science Co., Ltd. (PSS) launched MagDEA Dx SV, a nucleic acid extraction reagent, in October 2015 to meet the requirements of the genetic testing market. MagDEA Dx SV was designed and developed according to the regulations of the EU Directive 98/79/EC (IVD Directive). It allows to extract nucleic acids from 200 µL sample volumes on the geneLEAD XII plus, a fully automated genetic testing instrument, and on the magLEAD 6gC and magLEAD 12gC, two fully automated nucleic acid extraction instruments.

Recently, PSS has developed a dedicated protocol to extract nucleic acids from 400 µL, i.e., twice the original sample volume, for the magLEAD 6gC and magLEAD 12gC instruments.

This note reports the performance of nucleic acid extraction from 400 µL sample volumes with the newly developed protocol using MagDEA Dx SV, a nucleic acid extraction reagent for fully automated systems, and magLEAD 6gC and magLEAD 12gC, two fully automated nucleic acid extraction instruments manufactured by PSS.

〈Methods〉

(A) M13KO7, a DNA bacteriophage, and MS2, an RNA bacteriophage, were added to 200 or 400 µL saline solution. Nucleic acids were extracted from the solutions using the MagDEA Dx SV reagent on the magLEAD 12gC instrument. The elution volume for each sample was set at 50 µL. The extracted nucleic acids were analyzed using real-time PCR or RT-PCR.

(B) M13KO7 and MS2 phages were serially diluted in nuclease-free water to prepare the samples. Nucleic acids were extracted from the samples using MagDEA Dx SV and magLEAD 12gC. The elution volume for each sample was set at 50 µL. The extracted nucleic acids were analyzed using real-time PCR.

(C) Nucleic acids were extracted from 400 µL each of 62 Hepatitis B virus (HBV)-containing human serum samples and 51 health serum samples from healthy patients using MagDEA Dx SV and magLEAD 12gC. Virus quantification in the extracted nucleic acid samples was performed with a CE-IVD marked, real-time PCR reagent designed for HBV quantification. PCR was performed according to the reagent manufacturer's instructions. For comparison, nucleic acids were prepared with another company's extraction method (Company A), according to the manufacturer's instructions.

(D) Nucleic acids were extracted from 400 µL each of 58 Hepatitis C virus (HCV)-containing human serum samples and 58 control serum samples from healthy patients using MagDEA Dx SV and magLEAD 12gC. Virus quantification in the extracted nucleic acid samples was performed using a CE-IVD-marked, real-time RT-PCR reagent designed for HCV quantification. RT-PCR was performed according to the reagent manufacturer's instructions. For comparison, nucleic acids were prepared with another company's extraction method (Company A), according to the manufacturer's instructions.

〈Results〉

(A) For both M13KO7 and MS2 phages, the Ct value of extracts from 400 µL samples showed a difference of approximately 1 compared to the Ct value of extracts from 200 µL samples (**Table 1**). This shows that twice the volume of nucleic acids can be quantitatively extracted and detected in proportion with the increase in sample volume.

M13 (DNA phage)

Sample volume	200 µL	400 µL
Ct Mean (N=6)	33.69	32.42
Ct SD	0.33	0.25
ΔC_T (Ct_{SV200} - Ct_{SV400})	-	1.27

MS2 (RNA phage)

Sample volume	200 µL	400 µL
Ct Mean (N=6)	30.58	29.41
Ct SD	0.22	0.09
ΔC_T (Ct_{SV200} - Ct_{SV400})	-	1.17

Table 1 : Comparison of extraction volumes between different sample volumes

(B) For the M13KO7 phage, the detection limit was 200 copies in extracts obtained from 200 µL samples and 20 copies in extracts from 400 µL samples. For the MS2 phage, the detection limit was 1,000 copies in extracts obtained from 200 µL samples and 125 copies in extracts from 400 µL samples (**Table 2**).

It was observed for both phage samples, that the higher the sample volume, the wider the range in which Ct values were detected. This shows that the detection sensitivity increases by about eight to tenfold when the sample volume is doubled.

Copy / Sample	M13 Phage	
	Sample 200 uL	Sample 400 uL
200	6 / 6	6 / 6
20	5 / 6	6 / 6
10	2 / 6	5 / 6
5	2 / 6	3 / 6
2.5	1 / 6	3 / 6
2	0 / 6	2 / 6
0.2	0 / 6	1 / 6

Copy / Sample	MS2 Phage	
	Sample 200 μL	Sample 400 μL
10000	6 / 6	6 / 6
1000	6 / 6	6 / 6
500	4 / 6	6 / 6
250	4 / 6	6 / 6
125	5 / 6	6 / 6
100	1 / 6	4 / 6
10	0 / 6	0 / 6

Table 2 :Comparison of detection sensitivity between different sample volumes

(C) Both extraction methods showed a high correlation regarding the quantitative values determined from HBV-positive specimens. The coefficient of correlation compared to Company A's extraction method (A method) was 0.97 and the slope was 1.0 (**Fig. 1**). Furthermore, the positive and negative agreement rates were 97 and 98 percent, respectively (**Table 3**). Three samples showed differing determination results, suggesting that the analysis results were unstable because these specimens contained low copy numbers and the detected values were close to the cutoff level.

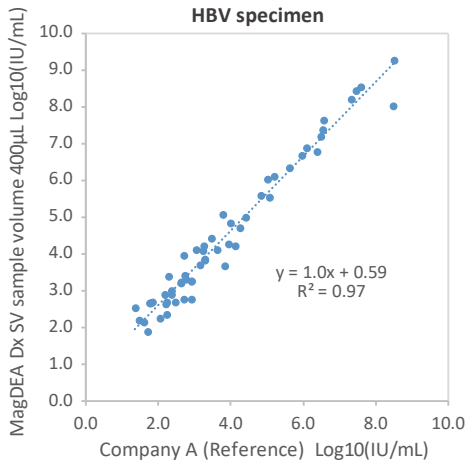

Fig. 1 : Correlation with a reference method (Company A) using HBV-containing serum samples

		Company A (Reference)		
		Positive	Negative	total
MagDEA Dx SV sample volume 400 μL	Positive	56	1	57
	Negative	2	53	55
	total	58	54	112

Table 3 : Results of the correlation test in HBV specimens (MagDEA Dx SV/ magLEAD 12gC vs. Company A)

(D) Both extraction methods showed high correlation regarding the quantitative values determined from HCV-positive specimens. The coefficient of correlation compared to Company A's extraction method was 0.98 and the slope was 0.97 (**Fig. 2**). Furthermore, the positive and negative agreement rates for Company A's extraction method were 100 percent (**Table 4**).

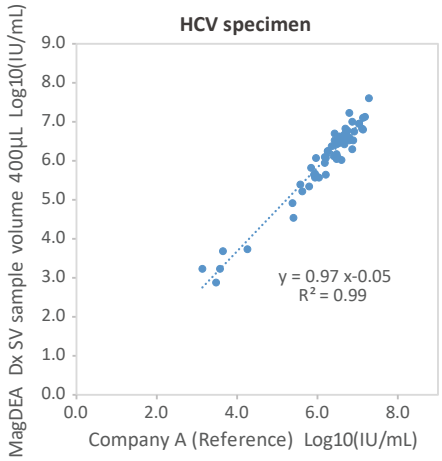

Fig. 2 : Correlation with a reference method (Company A) using HCV-containing serum samples

		Company A (Reference)		
		Positive	Negative	total
MagDEA Dx SV sample volume 400 μL	Positive	57	0	57
	Negative	0	59	59
	total	57	59	116

Table 4 : Results of the correlation test in HCV specimens (MagDEA Dx SV/ magLEAD 12gC vs. Company A)

〈Conclusion〉

MagDEA Dx SV, manufactured by PSS, was shown to successfully extract nucleic acids from 400 μL samples on two fully automated nucleic acid extraction instruments: magLEAD 6gC and magLEAD 12gC.

The results showed that the same reagent could be used with twice the sample volume by a simple protocol adjustment. Using this new protocol can improve the recovery rate and sensitivity in samples that are expected to contain low copy numbers of target nucleic acids.

〈Product information〉

Product Name	Product No.	Quantity
MagDEA Dx SV	E1300	48 tests

Devices

Product Name	Product No.	Consumables		
		Product Name	Product No.	Quantity
magLEAD 6gC	A1060	magLEAD Consumable Kit	F4430	50 tests
magLEAD 12gC	A1120			

Protocol (IC card)

Product Name	Sample volume	Correspondence instrument	Product No.
MagDEA Dx SV 200	200 μL	magLEAD 6gC	I-7706
		magLEAD 12gC	I-7712
MagDEA Dx SV 400	400 μL	magLEAD 6gC	I-7806
		magLEAD 12gC	I-7812

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