"MagDEA Dx SV," a pre-filled reagent for fully-automated nucleic acid extraction

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〈Introduction〉

Recent remarkable advances in gene analysis technology have allowed genetic studies and tests to be widely and readily performed. Genetic tests can be applied to various fields, such as oncology, infection diseases, pre-symptomatic diagnosis, prenatal diagnosis, aging, lifestyle-related diseases, and pharmacogenomics. Mainly in the infection and oncology fields, real-time PCR genetic testing, among other approaches, is used for definitive diagnosis, determination of treatment indications, therapeutic monitoring, and so on. It has thus become the main engine for the growth of the genetic test market. Generally, genetic testing consists of the following three steps: (1) extraction and purification of DNA/RNA from a sample, (2) setting up for PCR reactions of the extracted DNA/RNA, and (3) conducting PCR reactions and data analysis. In first step (nucleic acid extraction process) of these three, high quality DNA/RNA should be extracted and purified from various types of samples and used for real-time PCR assay to obtain high quality real-time PCR test results. In addition, in case of diagnosis of infectious diseases, detection of low copy number of bacterial and viral DNA/RNA is often important for sensitivity of diagnostic tests. Thus, the capability to extract/purify low amount of DNA/RNA (low copy number of DNA/RNA) in samples is also an important factor. Furthermore, shorter time from receiving the samples to reporting the diagnostic results is also an important factor. Therefore, expediting the extraction and purification steps is necessary.

At Precision System Science Co., Ltd. (PSS), we have developed a novel nucleic acid extraction reagent, "MagDEA Dx SV," to respond to the needs of the ever-expanding genetic testing market, and introduced it to the market in October 2015. MagDEA Dx SV was designed and developed as a nucleic acid extraction reagent set for in vitro diagnostics use in accordance with the EU Directive 98/79/EC (IVD Directive). It can be used in combination with fully-automated nucleic acid extraction devices, "magLEAD 6gC" and "magLEAD 12gC," and a fully-automated real-time PCR device, "geneLEAD XII plus." In this report, we will introduce the characteristics and performance of "MagDEA Dx SV."

〈Principle and methods for nucleic acid extraction〉

MagDEA Dx SV extracts and purifies nucleic acids using magnetic particles based chemistry. In automated devices, there are several methods of handling magnetic particles, but we use our original technology termed the "Magtration® method" **(Fig. 1)**. The Magtration method was designed to capture and disperse magnetic particles in a dispensing chip by applying a magnet on the side of the dispensing chip. Based on this principle, we at PSS have automated the nucleic acid extraction process.[1)]

As shown in **Fig. 2** , the nucleic acid extraction process consists of the following four steps: (1) lysis of proteins in a sample in the presence of chaotropic substances, (2) binding of DNA/RNA by the Van der Waals force, such as dipole-dipole interactions and hydrogen bonding, between phosphate residues deprived of hydration water by the chaotropic effect and magnetic carrier particles also similarly deprived of hydration water, (3) washing extraneous substances out of the magnetic particles to which nucleic acids are bound, and (4) elution of nucleic acids from the magnetic particles.[2)]

All reagents necessary for nucleic acid extraction are enclosed in a cartridge beforehand **(Fig. 3)**, making nucleic acid extraction possible with the combined use of this cartridge with consumables exclusive to the device **(Fig. 4)**.

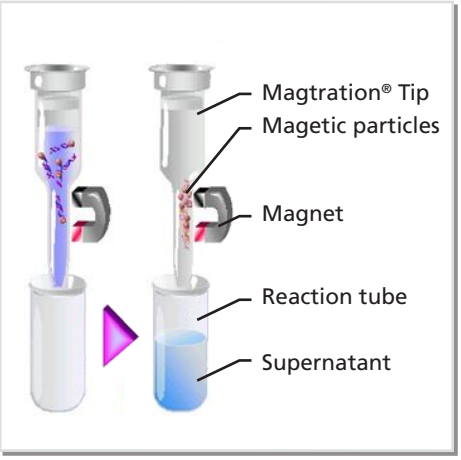


Fig. 1 : Magtration® Technology

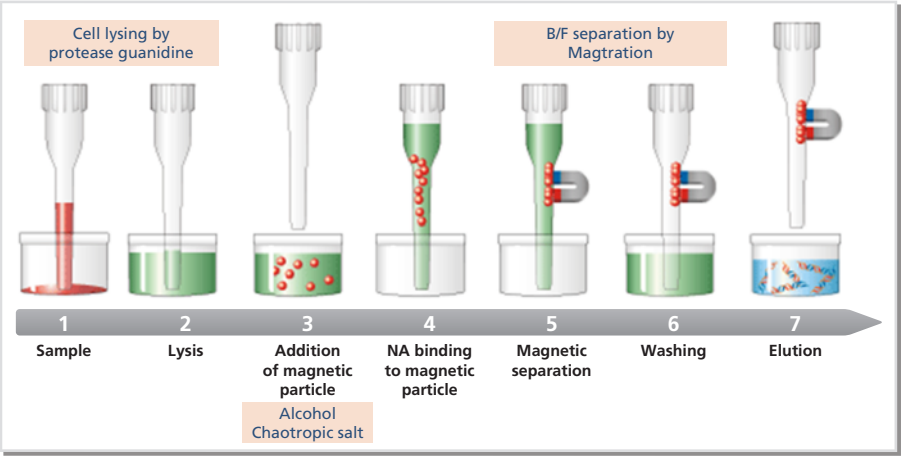


Fig. 2 : MagDEA Dx SV nucleic acid extraction work flow

Fig. 3 Reagent cartridge

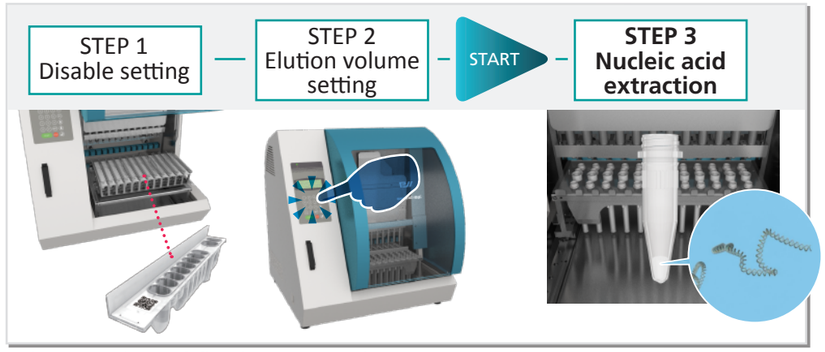


Fig. 4 Operation work flow (magLEAD 12gC)

⟨Nucleic acid extraction performance⟩

The table on the right summarizes the characteristics of the MagDEA Dx SV **(Table 1)**.

Using MagDEA Dx SV, extraction of total nucleic acids (DNA and RNA) from whole blood, plasma, serum, urine, cerebrospinal fluid [CSF], and swabs as shown on the right, can be performed with a single protocol (when whole blood is used as a sample, genomic DNA will be extracted). Performances of (1) genome DNA extraction from human whole blood and (2) viral DNA/RNA extraction are described below.

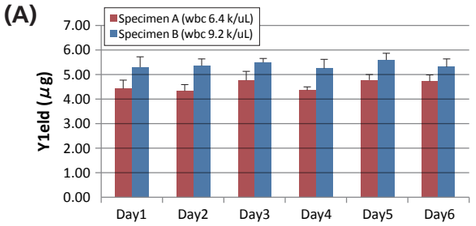
Table 1

Summary specifications for MagDEA Dx SV	
Extraction principle	Nucleic acid extraction method using magnetic particles
Sample volume	200 µL
Eluent volume	50, 100 or 200 µL
Extraction time	Approx.. 25 min
Sample type	Whole blood, serum, plasma, urine, CSF, swab
2D code information	Including reagent name, lot number, and expiration date information
Operation protocol	Single protocol for various samples types
Others (consumables)	Consumables kits for geneLEAD and for magLEAD are additionally required. An IC card for magLEAD 6gC/12gC is additionally required.
Storage conditions	2 years at room temperature (10–30°C)
Applicable instruments	geneLEAD XII plus, magLEAD 6gC, magLEAD 12gC

(1) Genomic DNA extraction from human whole blood

Genomic DNA was extracted from two types of human whole blood (with added EDTA or ACD as coagulants) with the setting of the sample volume at 200 µL and the extraction liquid volume at 50 µL. The observed absorbance ratios of 260nm/230nm and 260nm/280nm, which are indexes of the concentration and quality of the extracted genome DNA, are shown in **Fig. 5 (A)**. In addition, gel electrophoretic profiles of extracted genomic DNA were shown in **Fig. 5 (B)**. The yields of genome DNA differed depending on leukocyte counts in the samples. Reproducibility of the yield extracted from the same sample and the quality of the extract were both good. In addition, the electrophoresis results showed that DNA with a length of at least 20 kbp was recovered as a main fraction.

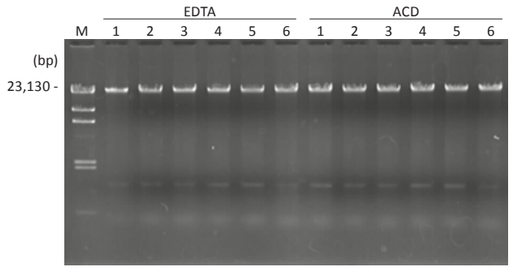
(A)



Specimen A (EDTA: wbc 6.4 k/µL)				Specimen B (ACD: wbc 9.2 k/µL)			
	Yield (µg)	SD	CV(%)		Yield (µg)	SD	CV(%)
Day 1	4.5	0.3	6.1	Day 1	5.2	0.4	6.8
Day 2	4.4	0.2	5.3	Day 2	5.0	0.3	6.6
Day 3	4.8	0.2	4.2	Day 3	5.3	0.2	4.5
Day 4	4.5	0.1	1.8	Day 4	5.0	0.4	8.0
Day 5	4.8	0.1	2.2	Day 5	5.5	0.2	4.1
Day 6	4.8	0.1	2.2	Day 6	5.1	0.3	6.1
Total	4.6	0.2	5.2	Total	5.2	0.3	6.6

	$A_{260/230}$	$A_{260/280}$
Specimen A	1.70 ± 0.11	1.88 ± 0.06
Specimen B	1.85 ± 0.17	1.89 ± 0.05

(B)



0.8% Agarose Gel

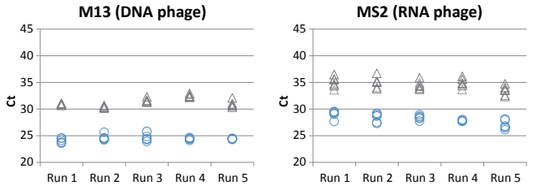
M: λ-*Hind* III digest marker
1 - 6: Extracted gDNA from six difference whole blood samples

Fig. 5 : Extraction of genomic DNA from whole blood using MagDEA Dx SV

(A) Genome DNA was extracted from two types of whole blood on 6 different days, and the yield of extracted DNA was measured.
(B) The extracted genomic DNA was analyzed by agarose gel electrophoresis. In addition, the absorbance ratios of the extracted nucleic acids were calculated.

(2) Extraction of viral DNA/RNA

Viral DNA/RNA was extracted using a phage sample as a viral model. M13KO7 phage was used as a DNA viral model, and MS2 phage was used as an RNA viral model. These were spiked into human serum (200 µL), and nucleic acids were extracted with an elution liquid (50 µL). Then, extraction efficiencies were evaluated by real-time PCR using genes of individual phages as targets. The extraction reproducibility data are shown in **Fig. 6**. Stable real-time PCR multiplication, independent of the number of copies, was obtained in 6 samples of the same run and in 5 different runs.



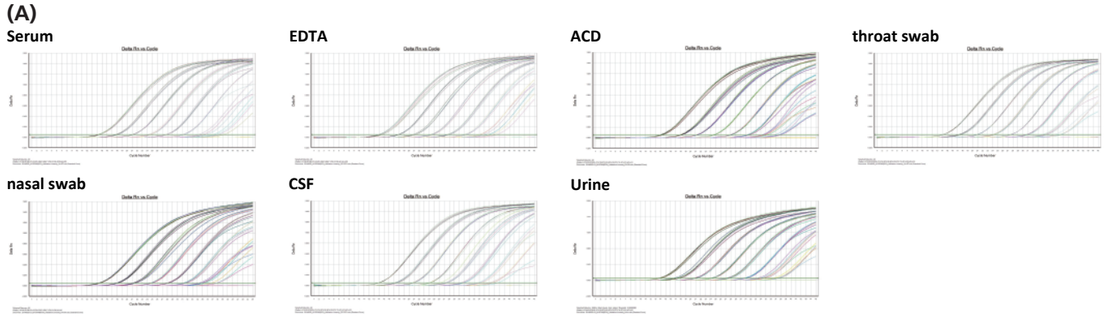
	M13 H 100cp/µL		M13 L 100cp/µL		MS2 H 100cp/µL		MS2 L 100cp/µL	
	Ct	SD	Ct	SD	Ct	SD	Ct	SD
Run 1	24.60	0.66	30.88	0.16	29.10	0.82	33.46	0.89
Run 2	24.05	0.42	30.37	0.18	29.08	0.12	35.04	0.69
Run 3	24.59	0.53	31.57	0.36	28.44	0.41	35.18	0.77
Run 4	24.42	0.08	32.58	0.32	28.42	0.77	34.51	1.02
Run 5	25.62	0.23	30.94	0.60	27.79	0.81	35.30	1.01

Fig. 6 : Reproducibility of extraction from the sera of viral models M13 and MS2

M13 and MS2 were spiked into individual sera to achieve the numbers of copies described in the Figure, and nucleic acids were extracted. After the extraction, the yields of M13 and MS2 nucleic acids were examined.

One of the strengths of MagDEA Dx SV is that it enables extraction of nucleic acids from various samples using a single reagent set and a single protocol. **Fig. 7** shows the extraction results from 7 types of sample matrices (serum, EDTA plasma, citric acid plasma, pharyngeal swab, nasal swab, CSF, and urine). In all sample matrices, linearity was high and PCR efficiencies were ≥95%. In addition, the real-time PCR results showed nearly the same Ct values, independently of the types of sample matrices.

(A)

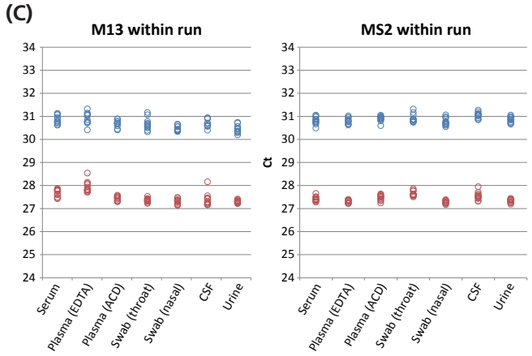


(B)

	Serum	Plasma (EDTA)	Plasma (ACD)	Swab (Throat)
Slope	-3.447	-3.406	-3.415	-3.369
	Pass	Pass	Pass	Pass
correlation coefficient (R^2)	0.995	0.997	0.998	0.999
	Pass	Pass	Pass	Pass
PCR efficiency (%)	95.027	96.594	96.253	98.061
	Pass	Pass	Pass	Pass
y-intercept	41.863	41.556	41.782	41.097

	Swab (nasal)	CSF	Urine
Slope	-3.391	-3.361	-3.397
	Pass	Pass	Pass
correlation coefficient (R^2)	0.999	0.996	0.998
	Pass	Pass	Pass
PCR efficiency (%)	97.215	98.405	96.954
	Pass	Pass	Pass
y-intercept	41.463	40.883	41.052

(C)



(D)

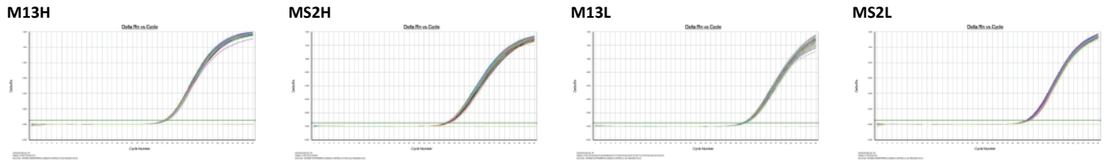


Fig. 7 : Results of extraction from different types of sample matrices

(A) M13 (from 10^8copies to 10^1 copies; 200 µL each) was spiked into different types of sample matrices, and the mixtures were extracted with an eluent (50 µL each). The resultant extracts were analyzed by real-time PCR. (B) The slope, linearity (R2), and PCR efficiency were calculated from the Ct values of real-time PCR obtained in (A). (C), (D) Two different concentrations of M13 and MS2 were added to various sample matrices, and the mixtures were extracted with MagDEA Dx SV. The resultant extracts were analyzed by real-time PCR.

Next, we compared the above method with other extraction methods using clinical samples. As a sample, we measured hepatitis B virus (DNA virus) (positive, 30; negative, 20). The results are shown in **Fig. 8**. Nucleic acids extracted with MagDEA Dx SV also correlated towel with the reference standard.

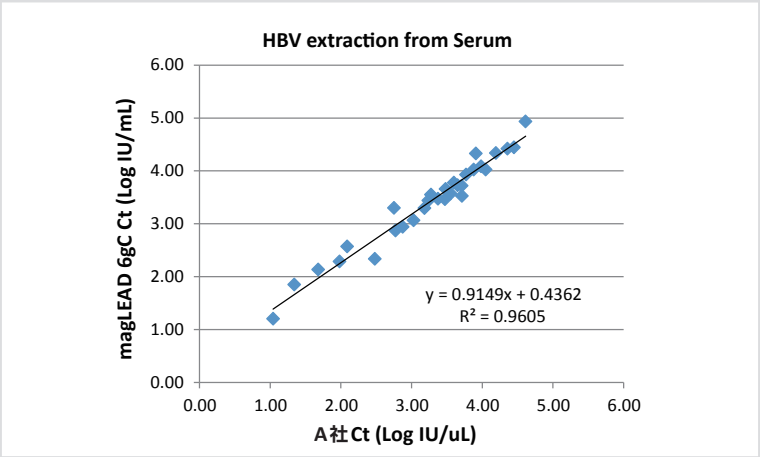


Fig. 8 : Performance of nucleic acid extraction from clinical samples

Nucleic acids were extracted with MagDEA Dx SV from sera obtained from HBV-infected persons or from healthy persons, and viruses were then assayed using a commercially available real-time PCR reagent for HBV assay. PCR was conducted following the instructions for use (IFU), and a sample of nucleic acids extracted by the method described in the IFU was used as a reference.

〈Summary〉

MagDEA Dx SV is a pre-filled DNA/RAN extraction/purification reagent developed for use with PSS' s fully-automated extraction devices, "magLEAD 6gC" and "magLEAD 12gC, and fully-automated genetic testing device, "geneLEAD XII plus." The reagent set can be used not only for extraction of a small amount of nucleic acids in a sample, which is necessary for the detection of low copy number of viral DNA/RNA by the real-time PCR method, but also for the recovery of a large amount of DNA, including genomic DNA from whole blood. In addition, because all necessary reagents are prepacked and because nucleic acid extraction from different sample matrices can be performed with a single reagent and a single protocol, MagDEA Dx SV is a reagent that can be flexibly applied to streamlining the laboratory workflow in laboratories where small amounts but many varieties of samples are used.

〈References〉

1) K.Obata et al, Development of a Novel Method for Operation Magnetic Prticles, Magtration Technology, and Its Use for Automating Nucleic Acid Purification, Jornal of Bioscience and Bioengineering, Vol.91, No.5, 500-503, 2001.

2) R.Boom et.al, Rapid and simple method for purification of nucleic acids., Journal of Clinical Microbiology, 28(3), 495-503, 1990.

〈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			
geneLEAD XII plus	A2603	geneLEAD Consumable Set	F2118	48 tests
		geneLEAD PCR Cassette	F2121	192 tests
		geneLEAD Waste Box	F2125	20 items

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