

## Extraction of norovirus from stool samples using MagDEA Dx SV

(Example of using “MagDEA Dx SV,” a prepacked reagent set for fully-automated nucleic acid extraction)

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### 〈Introduction〉

Norovirus causes acute gastroenteritis symptoms including vomiting and diarrhea in humans. It is known as a causative virus of “winter” viral gastroenteritis and food poisoning from which more people suffer between early fall and early spring. Oral (food, fecal-oral) is the main route of transmission to humans. The common sources of infection are: infected people’s feces and vomitus and things being contaminated directly or indirectly by their feces and vomitus, and foods that cause food poisoning (through eating raw or undercooked oysters and other bivalve shellfish contaminated by the virus, foods contaminated by infected people, etc.).

Reproducibility of norovirus is not good when grown in cell culture, and for this reason, basic research on norovirus has been delayed. However, in recent years, the total genome sequence has been determined for more than 20 norovirus strains, and the viral genomes have been analyzed in detail. As a result, a real-time RT PCR system targeting the most highly conserved region of the genomes was developed. This method enabled super-sensitive and quantitative measurement of norovirus genomes.

This note reports a comparison of norovirus extraction from pretreated stool samples using “MagDEA Dx SV,” a nucleic acid extraction reagent for fully automated systems, vs. “MagDEA DNA/RNA 200 virus (GC),” a reagent which has been conventionally used for norovirus genome extraction, both of which manufactured by Precision System Science Co., Ltd. (PSS).

### 〈Methods〉

A 10% emulsion of stool in PBS (pH 7.2-7.6) was centrifuged at 15,000 rpm for 5 minutes and the resulting supernatant was collected. The stool samples used were provided by the Osaka Prefectural Institute of Public Health.

Model	Magtration System 12GC PLUS	magLEAD 12gC
Reagent	MagDEA DNA/RNA virus (GC) Product number E7003	MagDEA Dx SV Product number E1300
Plastic consumables	Included in the reagent kit	magLEAD Consumable Kit Product number F4430
Protocol (IC card)	MagDEA DNA/RNA 200 virus	MagDEA Dx SV 200 12gC Ver.1.0
Extraction sample volume	200 µL	200 µL
Elution volume	50 µL	50 µL

Norovirus was quantitatively detected in the extracted nucleic acids using real-time PCR based on the attachment to Notice No. 0514004 from the Inspection and Safety Division, Department of Food Safety, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare, dated May. 14, 2007.

### 〈Results〉

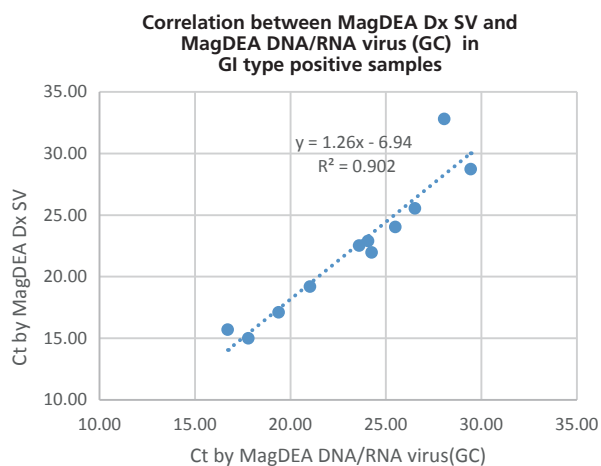
Nucleic acids were extracted from 49 stool samples using PSS’s products “MagDEA Dx SV” and “MagDEA DNA/RNA virus (GC).” **Table 1** and **Table 2** show the evaluation of extracted nucleic acids by real-time PCR. The sensitivity and specificity of “MagDEA Dx SV” for the detection of GI type norovirus in clinical samples was 100% compared to those of “MagDEA DNA/RNA virus (GC).” The sensitivity, specificity and concordance rate of “MagDEA Dx SV” for the detection of GII type norovirus in clinical samples were 95%, 96% and 98%, respectively. The shape of an amplification curve obtained by real-time PCR suggested that one sample which resulted in a different judgment might have been a positive sample with a low number of copies. Furthermore the Ct values of the extracted positive samples showed a high correlation between the two extraction reagents (**Fig.1, Fig.2**).

		MagDEA DNA/RNA virus (GC)		
		Positive	Negative	total
MagDEA Dx SV	Positive	11	0	11
	Negative	0	38	38
	total	11	38	49

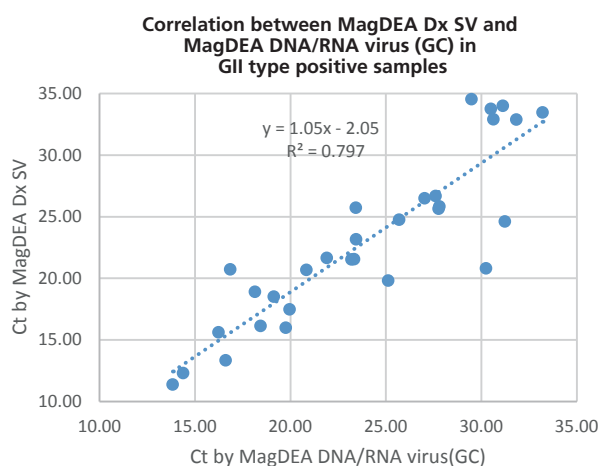
**Table 1 : Evaluation of performance for MagDEA Dx SV and MagDEA DNA/RNA virus (GC) extraction in clinical samples of GI type norovirus**

		MagDEA DNA/RNA virus (GC)		
		Positive	Negative	total
MagDEA Dx SV	Positive	30	1	31
	Negative	0	18	18
	total	30	19	49

**Table 2 : Evaluation of performance for MagDEA Dx SV and MagDEA DNA/RNA virus(GC) extraction in clinical samples of GII type norovirus**



**Fig. 1 : Correlation between MagDEA Dx SV and MagDEA DNA/RNA virus (GC) in GI type positive samples**



**Fig. 2 . Correlation between MagDEA Dx SV and MagDEA DNA/RNA virus (GC) in GII type positive samples**

## <Conclusion>

"MagDEA Dx SV" manufactured by PSS was capable of norovirus extraction from pretreated stool samples and real-time PCR detection as with "MagDEA DNA/RNA 200 virus (GC)."

## <References>

1) The Director of the Inspection and Safety Division, Department of Food Safety, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare, "Detection Method for Norovirus" attached to Notice No. 0514004 from the Inspection and Safety Division, Department of Food Safety, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare, dated May. 14, 2007.

## Contact details



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## <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