Extraction of influenza virus from nasopharyngeal mucosa 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〉

Influenza virus causes an infectious disease "influenza" when transmitted to humans. There are three types of influenza viruses: types A, B and C, and type A and B viruses are epidemically spread.

For patients with the acute phase, diagnosis is made by isolating the virus after infecting the amniotic cavity of an embryonated egg or inoculating cultured cells (MDCK cells) with a throat swab or a gargle fluid sample. Serological diagnostic methods include complement fixation (CF) method and hemagglutination inhibition (HI) test which require 2 to 3 weeks to give a definitive diagnosis, as the diagnosis cannot be given before the titer increases by four-fold of that in the acute and recovery phases. CF antibodies recognize internal viral antigens and are capable of differentiating influenza types A, B and C but not the subtypes of influenza type A virus. HI antibodies are detected for a long period of time after infection and are capable of typing, subtyping and measuring the extent of antigenic variation relatively easily. For these reasons, they are useful for seroepidemiological studies and vaccine effectiveness studies. In contrast, a genetic diagnostic method (RT-PCR) is useful for rapid diagnosis, has higher sensitivity compared to antibody testing, and is capable of typing and subtyping.

This note reports a comparison of influenza virus extraction from nasopharyngeal mucosa 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 the extraction of influenza virus genomes, both of which manufactured by Precision System Science Co., Ltd. (PSS).

〈Methods〉

A cotton swab used in nose or throat swabbing was dipped in a Hanks' balanced salt solution. It was centrifuged at 9,000 rpm for 10 minutes and the nucleic acid was extracted from the resulting supernatant. The nasal and throat swab samples were provided by the Osaka Prefectural Institute of Public Health.

The reagents and the conditions used for the extraction were as follows:

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

Influenza virus was extracted from nucleic acids using real-time RT PCR in accordance with "Manual on Influenza Diagnosis (Third Edition)" issued by the National Institute of Infectious Diseases, Japan.

〈Results〉

Nucleic acids were extracted from a total of 50 samples using PSS's reagents "MagDEA Dx SV" and "MagDEA DNA/RNA virus (GC)." **Table 1** and **Table 2** show the results of analysis and comparison using real-time PCR. The type A (for detection of type A M gene) and type B (for detection of type B NS gene) influenza virus positive/negative samples determined by "MagDEA Dx SV" were consistent with those of "MagDEA DNA/RNA virus (GC)" **(Table 1, Table 2)**. Furthermore 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	20	0	20
	Negative	0	30	30
	total	20	30	50

Table 1 : Evaluation of influenza Type A virus extraction performance of MagDEA Dx SV and MagDEA DNA/RNA virus (GC) in nasal or throat swab samples

		MagDEA DNA/RNA virus (GC)		
		Positive	Negative	total
MagDEA Dx SV	Positive	22	0	22
	Negative	0	28	28
	total	22	28	50

Table 2 : Evaluation of influenza Type B virus extraction performance of MagDEA Dx SV and MagDEA DNA/RNA virus (GC) in nasal or throat swab samples

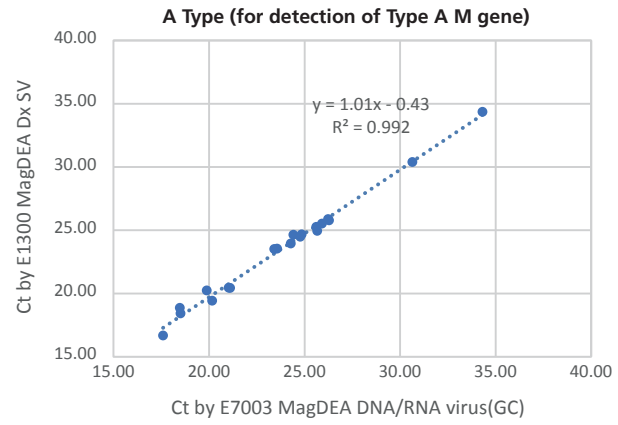

Fig. 1 : Correlation between MagDEA Dx SV and MagDEA DNA/RNA virus (GC) in influenza Type A virus positive samples

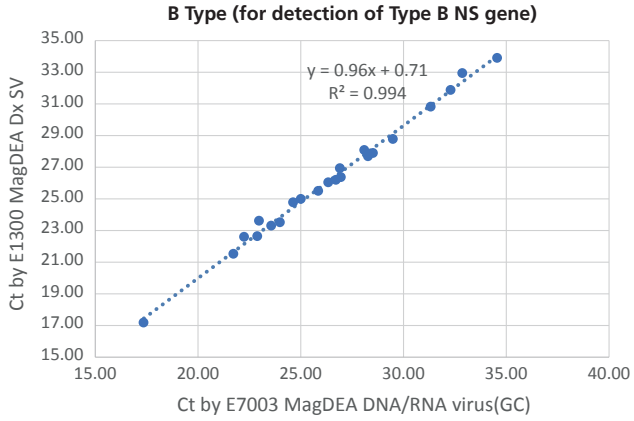

Fig. 2 : Correlation between MagDEA Dx SV and MagDEA DNA/RNA virus (GC) in influenza Type B virus positive samples

〈Conclusion〉

"MagDEA Dx SV" manufactured by PSS was capable of influenza virus extraction from nasopharyngeal mucosa samples and real-time PCR detection was possible, as with "MagDEA DNA/RNA 200 virus (GC)."

〈References〉

1) "Manual on Influenza Diagnosis (Third Edition)" (National Institute of Infectious Diseases, Japan)

〈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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