Extraction of adenovirus from stool and throat swab samples using MagDEA Dx SV

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

IVD Reagent Development Department,
LSM Business Division,
Precision System Science Co., Ltd.

〈Introduction〉

Adenovirus is a double-stranded DNA virus with an icosahedral, non-enveloped structure. In recent years, it has been reported as a genotype determined by the complete genome sequence and there are presently more than 67 adenovirus genotypes.

It causes various clinical symptoms of: respiratory diseases including pharyngitis, tonsillitis and pneumonitis; ophthalmologic diseases including pharyngoconjunctival fever and epidemic keratoconjunctivitis; gastrointestinal diseases such as gastroenteritis; and urologic diseases such as hemorrhagic cystitis. Additionally, those of adenovirus-related hepatitis, pancreatitis, and cerebritis have also been reported. Approximately 10% of infantile acute respiratory infections are said to be caused by adenovirus infections, and acute respiratory infections in children tend to become severe. Adenovirus is therefore considered a major pathogen.

A definitive diagnosis is made by the isolation of the virus from materials including rhinorrhea, saliva, sputum, stool, swab, wash specimens, pleural fluid, and cerebrospinal fluid. It can also be made by the detection of viral antigens using immunochromatography test kits and enzyme-linked immunosorbent assay (ELISA) method using antigen detection kits which are commercially available and generally used for early diagnosis, but serotypes cannot be determined by these methods. In contrast, PCR-based molecular typing methods is useful for rapid diagnosis and has recently been performed as a simple and rapid method.

This note reports a comparison of adenovirus extraction from pretreated stool and throat swab 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 adenovirus genomes, both of which manufactured by Precision System Science Co., Ltd. (PSS).

〈Methods〉

Each sample was pretreated by the method shown below. Stool and throat swab samples were provided by the Osaka Prefectural Institute of Public Health.

A. Stool
A stool sample was added to PBS (pH 7.2-7.6) to make a 10% emulsion. It was then centrifuged at 15,000 rpm for 5 minutes and the supernatant was recovered.

B. Throat swab
A cotton swab used in nasal or throat swabbing was dipped in a Hanks' balanced salt solution. It was centrifuged at 15,000 rpm for 5 minutes and the supernatant was recovered.

Nucleic acids were extracted from 200 µL of fluid recovered from A and B under the following conditions:

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

Adenovirus was detectedfrom nucleic acids using real-time PCR in accordance with "Manual on examinations and diagnosis of pharyngoconjunctival fever and epidemic keratoconjunctivitis (Second Edition)" issued by the National Institute of Infectious Diseases, Japan.

〈Results〉

Nucleic acids were extracted from a total of 40 samples including 13 stool and 27 throat swab samples using PSS's reagents "MagDEA Dx SV"and "MagDEA DNA/RNA virus (GC)" and were analyzed and compared using real-time PCR **(Table 1)**. The sensitivity and specificity of "MagDEA Dx SV" in the stool samples were 100% and 80%, respectively, while those in the throat swab samples were 100% compared to "MagDEA DNA/RNA virus (GC)." The melting peak results obtained from real-time PCR suggested that one sample (*) which had different results might have been a negative sample. Furthermore Ct values of the extracted positive samples showed a high correlation between the two extraction reagents. **(Fig. 1)**

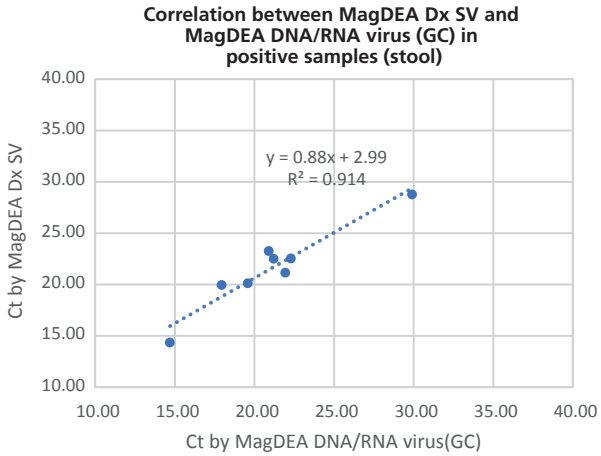
・Stool

		MagDEA DNA/RNA virus (GC)		
		Positive	Negative	total
MagDEA Dx SV	Positive	8	0	8
	Negative	1	4	5
	total	9	4	13

・Throat swab

		MagDEA DNA/RNA virus (GC)		
		Positive	Negative	total
MagDEA Dx SV	Positive	21	0	21
	Negative	0	6	6
	total	21	6	27

Table 1 : Evaluation of adenovirus extraction performance of MagDEA Dx SV and MagDEA DNA/RNA virus (GC) in stool and throat swab samples



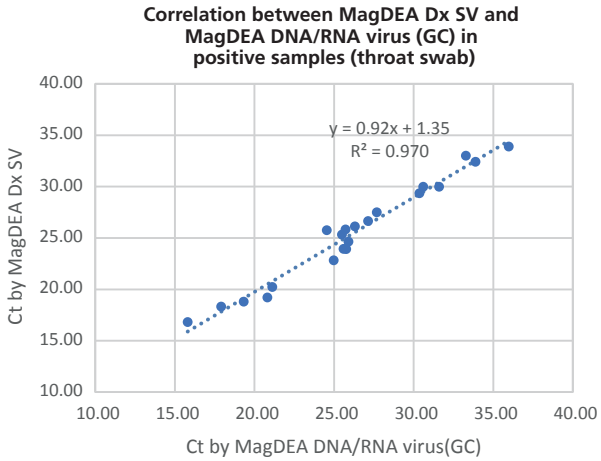


Fig. 1 : Correlation of values between MagDEA Dx SV and MagDEA DNA/RNA virus (GC) in adenovirus positive samples

〈Conclusion〉

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

〈References〉

1) "Manual on examinations and diagnosis of pharyngoconjunctival fever and epidemic keratoconjunctivitis (Second 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

Contact details

● For customers in Asia / Pacific
Precision System Science Co., Ltd.
URL: http://www.pss.co.jp
e-mail: service@pss.co.jp

● For customers in North / South America
Precision System Science USA, Inc
URL: http://www.pssbio.com
e-mail: contact@pssbio.com

● For customers in Europe / Africa / Middle East
Precision System Science Europe GmbH
e-mail: contact-psse@pss.co.jp