Extraction of dengue virus from Clinicalserum samples using MagDEA Dx SV

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

BioMolecular Laboratory,
N Health, Co., Ltd.
Bangkok Thailand

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

〈Introduction〉

Dengue fever and dengue hemorrhagic fever are infections caused directly by "dengue virus," which is transmitted by mosquitoes including Aedes aegypti. Dengue virus infections are found in tropical and subtropical areas where mosquitoes which transmit the virus exist. Dengue virus infections are common, especially in Southeast Asia, South Asia, South and Central America and the Caribbean. They have also been observed in Africa, Australia, China and Taiwan, and in recent years, in Japan.

Dengue virus, which belongs to a family Flaviviridae which is the same as Japanese encephalitis virus, is classified into four serotypes (types 1, 2, 3 and 4). The main diagnostic methods are (1) pathogen diagnosis and (2) serum diagnosis. Pathogen diagnosis includes detection of viral genes in blood using the RT-PCR method, detection of nonstructural protein 1 antigen (NS1 antigen) and virus isolation from mosquito-borne C6/36 cells, BHK cells and Vero cells. Furthermore, it enables genotyping through viral gene detection using genotype-specific primers. Serum diagnosis includes detection of IgM antibodies by the IgM capture ELISA.

This note reports the results of extracting dengue viral genes from clinical samples (serum, plasma, CSF) using "MagDEA Dx SV," a nucleic acid extraction reagent for fully automated systems manufactured by Precision System Science Co., Ltd. (PSS).

〈Methods〉

Nucleic acids were extracted from 200 µL of serum from patients who were infected with dengue virus using a fully automated nucleic acid extraction system "magLEAD 12gC," and a nucleic acid extraction reagent "MagDEA Dx SV," manufactured by PSS and a nucleic acid extraction instrument manufactured by another company (company X). Dengue virus was detected from the extracted nucleic acids using real-time PCR Simplexa™ Dengue (Focus Diagnostics, Inc., Code MOL3100) and 3M™ Integrated Cycler (Focus Diagnostics, Inc., Code MOL1001).

〈Results〉

Dengue virus was detected by Simplexa™ Dengue from 6 infected serum samples containing dengue virus of each serotype following nucleic acids extraction by MagDEA Dx SV and another company's nucleic acid extraction instrument (company X). **Fig. 1** shows real-time PCR Ct values. The nucleic acids were extracted from all of the serotypes with MagDEA Dx SV, and the Ct values were lower compared to those assessed by the company X's product.

Sample No.	Serotype	Company X	MagDEA Dx SV
1	3	16.2	14.6
2	1	19.5	17.8
3	4	28.1	24.9
4	2	15.2	14
5	2	19.2	16.3
6	1	27	25.4
7	4	37.4	29.6
8	3	37.5	29.7
9	4	38.4	31.1
10	3	36.4	29.6
11	4	36.5	35.4
12	4	38.8	33.8
13	1	39	34
14	4	37.4	32
15	3	36.4	26
16	4	37.8	29.3
17	4	37.4	36.5
18	3	38.6	27.9
19	4	39	34.6
20	4	37.5	33.7
21	4	39.5	34.5
22	4	39.3	38.2
23	4	39.6	37.7
24	3	38.9	29.9

Fig. 1 : Results of extraction for each serotype

Dengue virus was detected using Simplexa™ Dengue after the infected serum samples of each serotype's dengue virus were diluted with uninfected serum in serial tenfold steps followed by nucleic acids extraction from each diluted serum using MagDEA Dx SV. **Fig. 2** shows real-time PCR Ct values. Detection sensitivity differed depending on the serotypes, while the Ct values being calculated according to the dilution ratios showed a good linearity in all of the serotypes.

[Ct]

dilution Factor	Serotype 1		Serotype 2		Serotype 3		Serotype 4	
	n1	n2	n1	n2	n1	n2	n1	n2
1/1	16.1	16.5	14.3	14.1	14.2	14.8	19.0	19.0
$1/10^1$	19.4	20.2	17.6	17.8	17.9	18.4	22.8	22.6
$1/10^2$	24.2	23.6	21.2	20.9	21.7	21.9	26.1	26.1
$1/10^3$	27.3	26.6	24.6	24.3	25.2	25.5	29.5	29.4
$1/10^4$	30.7	30.7	27.9	28.0	28.9	28.9	33.3	33.2
$1/10^5$	34.3	34.2	32.0	31.4	32.3	32.4	37.3	38.9
$1/10^6$	37.0	37.1	35.6	ND	34.5	36.2	ND	39.2
$1/10^7$	39.1	39.4	ND	ND	39.6	ND	ND	ND
Slope	-3.4	-3.3	-3.6	-3.4	-3.5	-3.5	-3.6	-3.6
R^2	0.996	0.998	1.000	1.000	0.998	1.000	0.999	0.992
PCR Efficiency	98	99	91	95	92	92	89	90

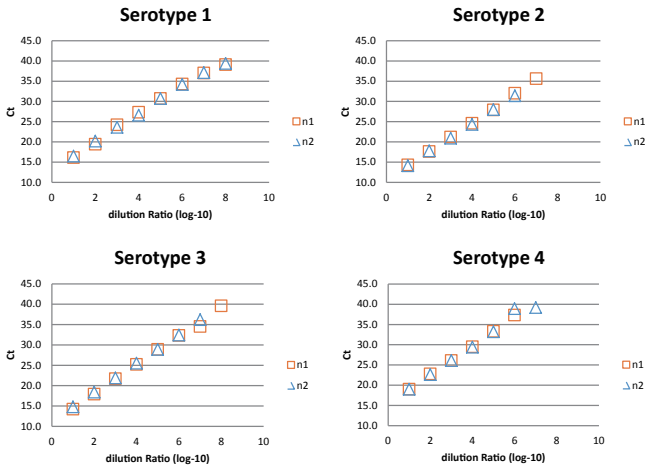

Fig. 2 : Results of extraction using diluted infection samples

Nucleic acids were extracted from 58 clinical samples (31 positive and 27 negative samples) using MagDEA Dx SV and company X's product and subsequently, dengue virus was detected using Simplexa™ Dengue. **Fig. 3** shows real-time PCR Ct values. A good correlation was found between MagDEA Dx SV and company X's product, and a 100% concordance rate was obtained in the results. The 31 positive samples included: 3 serotype 1 samples, 8 serotype 2 samples, 6 serotype 3 samples and 14 serotype 4 samples.

		Company X	
		Positive	Negative
MagDEA Dx SV	Positive	31	0
	Negative	0	27

Fig. 3 : Results of extraction using clinical samples

〈Conclusion〉

MagDEA Dx SV manufactured by PSS was capable of dengue virus extraction from serum samples and real-time PCR detection.

〈References〉

1) WHO, Dengue Guidelines for Diagnosis, Treatment, Prevention and Control, New edition (2009) WHO/HTM/NTD/DEN/2009.1

2) CDC http://www.cdc.gov/denge/epidemiology/index.html

〈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