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Newly Developed TaqMan Assay to Detect West Nile Viruses in a Wide Range of Viral Strains

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In the United States during 2003, 9,862 people were reported to be infected with West Nile virus (WNV) to the Centers for Disease Control and Prevention, and 264 of these people died of related encephalitis and meningitis (http://www.cdc.gov/ncidod/dvbid/westnile/surv&control CaseCount03_detailed.htm). There is the possibility of WNV infection even in Japan in the near future.

Genetically, WNV can be divided into two lineages. NY99, prevalent in the United States in a 1999 survey of avian and mosquito samples, belongs to lineage 1 (1). Lanciotti et al. (2) developed a TaqMan Reverse Transcriptase (RT)-PCR assay based on the sequence of the NY99 strain. The 3′NC primers and probe assay detects 0.1 PFU/5 μl of sample of NY99 strain and reacts with other six WNV strains. Two main WNV strains, g2266 (lineage 1) and FCG (lineage 2), are available in local public laboratories in Japan. However, they are genetically distinct from the NY99 strain. The 3′NC primers and probe assay cannot detect the FCG and g2266 strains used as respective control strains in real time polymerase chain reaction (real time PCR) in a Japanese local laboratory. It is thus necessary to develop new primers and probe sets to detect various WNV strains in order to screen avian and mosquito samples.

We developed a new TaqMan RT-PCR assay to detect both

Table 1. Newly developed oligonucleotide primers and probes used in the TaqMan assay

Primer	Genome position[1)]	Sequence (5′-3′)
WNV cap-forward	110-127	CAGGAGGGCCCGGYAARA
WNV cap-reverse	179-162	ATCAAGGACAAYMCGCGG
WNV cap-probe	129-154	FAM-CCGGGCTGTCAATATGCTAAAACGCG-TAMRA

[1)]: Genome position according to WNV NY99 complete sequence (GenBank accession number AF196835).

Table 2. Summary of sensitivity and specificity in West Nile viruses using cap primers and probe

	Sample	Sensitivity limit[1)]	
		cap primers and probe (PFU/tube)	3′NC primers and probe
WNV	NY99	10~100	0.1
	Eg101	5.4~54	Pos
	Kunjin (OR393)	200	Pos
	FCG	2.8×10^4~2.8×10^5	Neg
	g2266	Pos	Neg
JEV type 1	JEV/sw/Kagawa/24/2002	Neg	
	JEV/sw/Mie/41/2002	Neg	
	JEV/sw/Shizuoka/33/2002	Neg	
JEV type 3	JaGAr01	Neg	
	Nakayama	Neg	
	Beijing	Neg	
Dengue virus	type 1 (Hawaii)	Neg	
Dengue virus	type 2 (New Guinea C)	Neg	
Dengue virus	type 3 (H87)	Neg	
Dengue virus	type 4 (H241)	Neg	

[1)]: Pos shows positivity of TaqMan RT-PCR by qualitative testing using seed virus.

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lineages 1 and 2 WNVs including strains of NY99, Eg101, Kunjin (OR393), g2266, and FCG. To develop the TaqMan primers and probe, we performed multiple alignments using 12 WNV complete sequences (NY99, IS-98 STD, RO97-50, Italy-98, FCG, etc.) submitted to GenBank and used the nucleocapside (cap) coding region, which is highly conserved. Primers and probe are shown in Table 1, and the length of these amplicons was 70 bp. We also confirmed the specificity of this assay by using negative controls of Japanese encephalitis virus and dengue virus, respectively (Table 2).

To estimate the sensitivity of this assay, we performed 10-fold serial dilution samples of NY99, Eg101, Kunjin, and FCG strains using the WNV cap probe and primers. Table 2 shows the sensitivity of our cap primers and probe set. Compared with that of the 3´NC primers and probe, our cap primers and probe has a lower sensitivity to the NY99 strain. Further, our cap primers and probe detected a wider range of strains than did the 3´NC primers and probe, and has sufficient sensitivity for use in WNV-screening of field collected avian and mosquito samples. Our cap primers and probe will be useful for WNV surveillance.

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