Evaluation of the modified serotyping-NS1-ELISA to detect dengue NS1 and identify dengue serotypes in patient specimens

Dengue serotyping by NS1 ELISA

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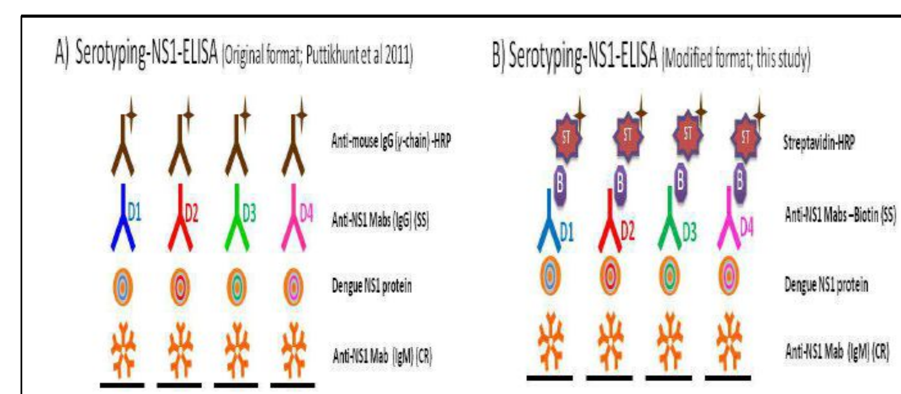
Introduction and purposes

Dengue virus (DENV), the cause of the mosquito-borne disease dengue hemorrhagic fever (DHF), consists of four serotypes (DENV1, DENV2, DENV3 and DENV4) that co-circulate in endemic areas. Severe DHF has been associated with a secondary response to an infection by heterotypic viruses. Recently, detection of DENV non-structural protein 1 (NS1) in patient blood become widely used as one of early laboratory diagnostics for DENV infection over the sophisticated RT-PCR which is still required as a standard method for dengue serotyping. In 2011, our group had first established the "Serotyping-NS1-ELISA" to detect DENV NS1 and to identify DENV serotypes simultaneously (Fig.1A). The assay was simplified and could predict 100% accurate for DENV1, DENV3 and DENV4. However, moderate sensitivity and serotyping errors were found in some specimens especially those from DENV2 infections. (Puttikhunt *et al.* (2011). Journal of Clinical Virology 50: 314-319).

This study therefore aimed to modify the new generation of serotyping-NS1-ELISA in order to enhance overall sensitivity and the accuracy of dengue serotyping for all four serotypes.

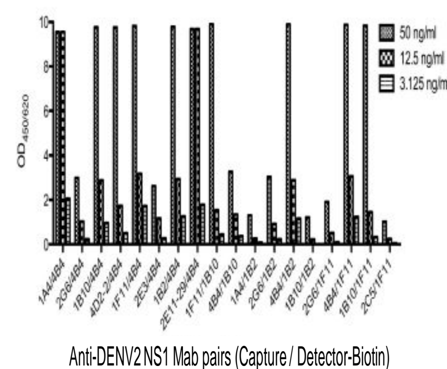
Fig. 1 Schematic diagram of "Serotyping-NS1-ELISA"



Selection of anti-DENV2 NS1 Mabs

The Serotyping-NS1-ELISA comprises of 4 pairs of serotype-specific anti-NS1 Mabs (IgG) which share a capture dengue cross-reactive Mab (IgM). To improve DENV2 serotype accuracy, a panel of anti-DENV2 NS1 Mabs were selected for the best antibody pair in a sandwich ELISA format. The detection Mabs were biotinylated to minimize the limitation of immunoglobulin isotype and the complex was revealed by HRP-conjugated streptavidin (Fig.2).

Fig. 2. Selection of anti-DENV2 NS1 Mabs

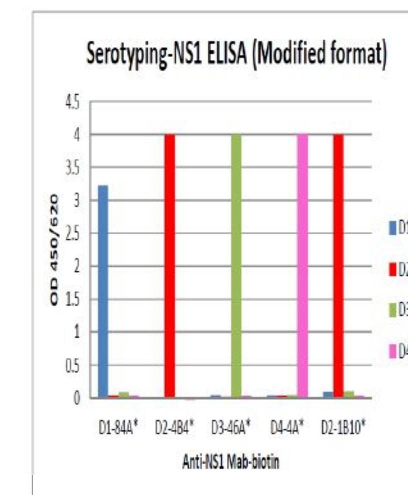


Among several pairs of anti-NS1 Mabs, 4B4 was first selected as a detection antibody according to its highest reactivity to DENV2 NS1. 1B10 and 1B2 could be optionals. The selected anti-DENV2 Mabs were used to replace an original anti-DENV2 Mab (3D1), whereas anti-NS1 Mabs for other serotypes were still the same as in the original ELISA format, but all serotype-specific antibodies were biotinylated. (Fig.1B)

Serotype-specificity of the assay

The biotinylated serotype-specific anti-NS1 Mabs of each serotype were used to separately detect NS1 of four serotypes which were previously captured by a coating dengue cross-reactive anti-NS1 Mab (IgM) in ELISA wells, followed by HRP-conjugated Streptavidin. The results demonstrated that those serotype-specific anti-NS1 Mabs detected only NS1 of homologous serotypes, but no cross-reactive to others (Fig. 3). The detection limit of the modified assay to NS1 of each serotype was lower than 1 ng/ml. *(compared with 1-15 ng/ml in the original assay)*

Fig. 3. Serotype-specificity of anti-NS1 Mabs



• OD reading at 4 indicated the over range of the reactivity
• NS1 antigen concentration was 50 ng/ml

Evaluation of the modified assay in clinical samples

Retrospective clinical samples (n=245) from a dengue hospital cohort in Thailand were used to evaluate the assay. Confirmation of dengue infected cases (n=195) and serotype identification were done by RT-PCR. Other febrile illness (OFI), non-dengue cases (n=50) were used as negative controls. The sample dilutions ranged from 1:3 to 1:10.

With four pairs of antibodies,145 of 195 dengue infected samples were positive by either one of four. In addition to DENV2-specific Mab 4B4, 1B10 increased 11 more positive cases of DENV2, probably caused by different epitope recognition. Thus, overall sensitivity of the assay was raised up to 80% (156/195) *(from 76.5% in the original assay)*. Sensitivities to each serotype were increased to 74%, 82%, 82.2% and 82% *(compared with 73.9%, 63.6%, 75% and 80% in the original assay)* for DENV1 to DENV4, respectively. The assay specificity was 100% as no false positive was found in all non-dengue OFI specimens. Importantly, identification of DENV serotypes of those positive samples by our modified assay was 100% accurate for all four serotypes (Table 1). *(improving from 82.4% DENV2-serotype accuracy of the original one)*

Table 1. Evaluation of the modified "Serotyping-NS1-ELISA"

		RT-PCR (Std)					Total	Serotype accuracy (%)
		DENV1	DENV2	DENV3	DENV4	Neg		
Modified Serotyping-NS1 ELISA (test)	DENV1	37	0	0	0	0	**37**	37/37 (100)
	DENV2	0	30+11	0	0	0	**41**	41/41 (100)
	DENV3	0	0	37	0	0	**37**	37/37 (100)
	DENV4	0	0	0	41	0	**41**	41/41 (100)
	Neg	13	9	8	9	50	**95**	
	Total	**50**	**50**	**45**	**50**	**50**	**245**	
Sensitivity to serotype (%)		37/50 (74)	41/50 (82)	37/45 (82.2)	41/50 (82)			

% Sensitivity (overall): [(37+41+37+41)/(50+50+45+50)] x100 = (156/195)x100 = 80%

% Specificity: (50/50)x100 = 100%

Conclusion: Direct detection of dengue serotypes in patients plasma by the "Serotyping-NS1-ELISA" overcomes the complexity of pre-processing specimens prior to serotyping by conventional RT-PCR. This simple platform is useful for dengue diagnosis and epidemiological studies for which DENV serotype data is necessary.

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