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Evaluation of a West Nile Virus Immunoglobulin A Capture Enzyme-Linked Immunosorbent Assay

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An in-house-developed enzyme-linked immunosorbent assay detected West Nile virus (WNV) immunoglobulin A (IgA) in 65 of 68 sera from WNV-infected patients; 40 of 63 WNV IgM-positive, IgG-negative serum or plasma specimens; 65 of 67 WNV IgM-positive, IgG-positive specimens; 0 of 70 WNV IgM-negative, IgG-negative specimens; and 0 of 64 archived blood donation sera. WNV IgA is thus highly prevalent among WNV-infected patients and typically appears after WNV IgM but before WNV IgG.

West Nile virus (WNV) immunoglobulin M (IgM) is usually detected in serum and plasma samples from infected patients at clinical presentation and remains detectable for well over a year in some patients (1, 8, 11). This persistence of WNV IgM has raised concerns about its clinical utility in geographic areas where WNV has been endemic for more than one season. A laboratory marker is needed to distinguish WNV IgM-positive patients infected in prior seasons from WNV IgM-positive patients infected during the present season. It has been suggested that WNV IgA may be such a marker (6). This idea is based on published findings for antibody responses following infection or vaccination with other flaviviruses related to WNV. Dengue virus-specific IgA appears quickly following infection and then falls to undetectable levels within a few months (5, 12); similarly, IgA induced by yellow fever virus vaccination falls to undetectable levels by about 80 days after vaccination (7). These trends, however, do not appear to characterize all flavivirus-induced antibody responses; 50% of children infected with Japanese encephalitis virus still had detectable virus-specific IgA at day 145 of follow-up (2). In order to investigate the value of WNV IgA as a discriminating marker for recent versus past WNV infection, we plan to test serial samples from WNV-infected individuals for WNV IgM, IgG, and IgA. This report describes the WNV IgA enzyme-linked immunosorbent assay (ELISA) developed for use in these planned studies.

WNV IgM and IgG detection. Some serum and plasma samples used to evaluate the WNV IgA ELISA were selected on the basis of WNV IgM and IgG results. These results were determined using ELISA kits from Focus Technologies (Cypress, Calif.). Assays were performed per the package inserts (3); samples positive for WNV IgM were retested using the background subtraction method to identify false-positive reactivity caused by heterophile antibodies (3, 10).

WNV IgA assay. The WNV IgA capture ELISA was patterned after the Focus Technologies WNV IgM capture ELISA (3, 9). PolySorp microtiter wells (Nunc, Roskilde, Denmark) were coated with rabbit anti-human IgA (alpha-chain specific; Jackson Immunoresearch, West Grove, Pa.) at 600 ng/well in 0.05 M carbonate buffer by overnight incubation at 4°C. The wells were then blocked for 2 h at room temperature (RT) with phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin (Serologicals Corp., Norcross, Ga.). Coated microtiter plates were air dried for 3 h, pouched with desiccant, and stored at 4°C until use. On the day of assay, wells were filled with wash buffer (PBS containing 0.05% Tween 20 [PBST]) and after 5 min the buffer was discarded. Serum or plasma diluted 1:101 in sample diluent (PBST containing 0.1% bovine serum albumin) was added to duplicate wells (0.1 ml per well), followed by a 1-h incubation at RT. After three washes, one well of each duplicate received 0.1 ml of sample diluent and the other well received 0.1 ml of recombinant WNV antigen (Focus Technologies) reconstituted per the WNV IgM ELISA kit package insert. After 2 h at RT, the wells were washed and then received 0.1 ml of horseradish peroxidase-conjugated 6B6C anti-flavivirus monoclonal antibody (Focus Technologies) diluted 1:300 in PBST. After 30 min at RT and after washing, wells received 0.1 ml of tetramethylbenzidine (enhanced K-blue; Neogen Corp., Lexington, Ky.); the color reaction was stopped after 30 min by adding 0.1 ml of 1 N sulfuric acid (Ricca Chemicals, Arlington, Tex.). Absorbance at 450 nm was measured using an ELISA reader (BioTek, Winooski, Vt.). All assays included a negative control, a positive control (pooled WNV IgM-positive, IgG-positive sera), and a calibrator specimen (pooled WNV IgM-positive, IgG-positive sera and WNV IgM-negative, IgG-negative

TABLE 1. Intra-assay and interassay variation data for the WNV IgA capture ELISA

Parameter	Value for:			
	Intra-assay comparison		Interassay comparison[a]	
	Positive control	Negative control	Positive control	Negative control
No. of determinations	9	9	15	15
Range of index values	6.24–7.31	0.66–0.86	5.10–6.78	0.59–0.87
Mean index	6.66	0.73	5.95	0.68
Standard deviation	0.31	0.07	0.50	0.09
Coefficient of variation	5%	10%	8%	13%

[a] Assays were performed over a 22-day period by using the same lot of IgA capture microtiter well plates.

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TABLE 2. WNV IgA results for various serum and plasma panels

Panel no.	Description of samples	n	No. (%) with WNV IgA index of:				
			<1.00	1.00–5.00	5.01–10.00	10.01–20.00	>20.00
1	WNV$^+$ by PRNT; collected July to September 2002	68	3 (4)	14 (21)	9 (13)	16 (24)	26 (38)
2	WNV IgM$^+$, IgG$^-$; collected August to September 2003	63	23 (37)	18 (29)	8 (13)	4 (6)	10 (16)
3	WNV IgM$^+$, IgG$^+$; collected August to September 2003	67	2 (3)	9 (13)	12 (18)	18 (27)	26 (39)
4	WNV IgM$^-$, IgG$^-$; collected August 2004	70	70 (100)	0 (0)	0 (0)	0 (0)	0 (0)
5	Los Angeles blood donor sera collected in 1999	64	64 (100)	0 (0)	0 (0)	0 (0)	0 (0)

sera). The net absorbance value was calculated for all samples by subtracting the absorbance value for the well receiving sample diluent from the absorbance value for the well receiving WNV antigen. Results were expressed as an index, calculated using the following formula: index = sample net absorbance value/calibrator net absorbance value. The calibrator was formulated to reflect the mean net absorbance plus three standard deviations for 31 WNV IgM-negative, IgG-negative specimens collected during a period of WNV inactivity (February 2004). An index of ≥1.00 was considered to indicate positivity.

WNV IgA assay precision. Intra-assay and interassay variation data for the WNV IgA assay positive and negative controls are shown in Table 1. Coefficient of variation values were <10% for the positive control and <15% for the negative control. Two lots of IgA capture wells were compared by testing 12 specimens (six WNV IgA negative, six WNV IgA positive) in parallel. Percent variance was calculated for each specimen by using the following formula: percent variance = [(lot 2 index − lot 1 index)/lot 1 index] × 100. The mean variance was 0% (range, −14 to +18%).

Evaluation of serum and plasma panels. Five serum or plasma panels were tested using the newly developed WNV IgA capture ELISA. Panels 1 to 3 were presumed to represent recent WNV infection, and panels 4 and 5 were included to assess assay specificity. Results for all five panels are summarized in Table 2. Panel 1 included 68 sera collected at the height of the 2002 WNV season (July to September) and confirmed as positive for WNV antibodies by Public Health Service Laboratories by using the plaque reduction neutralization test (PRNT) (10); 96% (65 of 68) of these samples were positive for WNV IgA. Panel 2 comprised 63 WNV IgM-positive, IgG-negative serum or plasma specimens collected from August to September 2003; due to the strong agreement between WNV IgM detection and positive PRNT results for samples from 2002 (10), we did not solicit Public Health Service Laboratories for PRNT results for WNV IgM-positive samples from 2003. The WNV IgM-positive, IgG-negative profile indicates that panel 2 samples were collected early in the WNV infectious process (4). WNV IgA was detected in 63% (40 of 63) of panel 2 samples; most WNV IgA-positive samples (26 of 40; 65%) had relatively low WNV IgA indexes (1.00 to 10.00). These findings are consistent with very recent infection and suggest that WNV IgA appears soon after WNV IgM but before WNV IgG. Panel 3 included 67 WNV IgM-positive, IgG-positive serum or plasma specimens also collected from August to September 2003. WNV IgA was present in 97% (65 of 67) of these samples, and most (44 of 67; 66%) had markedly elevated WNV IgA indexes (>10.00). Panel 4, containing 70 WNV IgM-negative, IgG-negative serum or plasma specimens collected during August 2004, was designed for assessment of the likelihood of WNV IgA detection in the absence of WNV IgM and IgG during a period of high WNV activity. All panel 4 samples were WNV IgA negative, indicating that detection of WNV IgA in the absence of WNV IgM and IgG is unlikely. Panel 5 included 64 sera collected from American Red Cross blood donors from the Los Angeles, Calif., area in 1999, well before WNV arrived in California; all panel 5 samples were WNV IgA negative.

Systematic studies assessing cross-reactivity in the WNV IgA assay due to St. Louis encephalitis virus (SLEV) infection, the other major flavivirus infection endemic in the United States, were not possible due to the unavailability of sufficient numbers of well-characterized samples. WNV IgA was not detected in four sera with a profile of past SLEV infection (SLEV PRNT titers at least fourfold higher than the WNV PRNT titer; SLEV IgG positive, IgM negative by indirect immunofluorescence). Further studies using samples from recently infected SLEV patients are required to assess the reactivity of SLEV IgA with WNV antigens.

These studies demonstrate that an alpha-capture ELISA effectively measures WNV IgA in human serum and plasma specimens. This sensitive and reproducible assay, along with previously described WNV IgG and IgM ELISA systems (3), is currently being used to characterize the timeline of WNV antibody production and persistence in WNV-infected individuals. Such data will help determine whether WNV IgA can serve as an effective laboratory marker for distinguishing recent from past WNV infection.

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