Journal of the American Mosquito Control Association, 19(4):440–444, 2003

SCIENTIFIC NOTE

SENSITIVITY OF THE VECTEST™ ANTIGEN ASSAY FOR EASTERN EQUINE ENCEPHALITIS AND WESTERN EQUINE ENCEPHALITIS VIRUSES

ROGER S. NASCI,[1] KRISTY L. GOTTFRIED,[1] KRISTEN L. BURKHALTER,[1] JEFFREY R. RYAN,[2,3] EVA EMMERICH[4] AND KIRTI DAVÉ[4]

ABSTRACT. VecTest™ assays for detecting eastern equine encephalitis virus (EEE) and western equine encephalitis virus (WEE) antigen in mosquito pools were evaluated to determine their sensitivity and specificity by using a range of EEE, WEE, St. Louis encephalitis virus (SLE), and West Nile virus (WN) dilutions as well as individual and pooled mosquitoes containing EEE or WEE. The EEE test produced reliable positive results with samples containing ≥ 5.3 $\log_{10}$ plaque-forming units (PFU) of EEE/ml, and the WEE test produced reliable positive results with samples containing ≥ 4.7 $\log_{10}$ PFU WEE/ml. Both assays detected the respective viral antigens in single virus-positive mosquitoes and in pools containing a single positive mosquito and 49 negative specimens. The SLE and WN assays also contained on the dipsticks accurately detected their respective viruses. No evidence was found of cross reaction or false positives in any of the tests. The VecTest assays were less sensitive than the EEE- and WEE-specific TaqMan reverse transcriptase polymerase chain reaction and Vero cell plaque assay, but appear to be useful for detecting arboviruses in mosquito-based arbovirus surveillance programs.

KEY WORDS Eastern equine encephalitis, western equine encephalitis, surveillance, VecTest, reverse transcriptase polymerase chain reaction, plaque assay, mosquito, vector

The VecTest™ antigen assays (Medical Analysis Systems, Camarillo, CA) are commercially available, 1-step, qualitative, dipstick-format, immunochromatographic tests that use type-specific monoclonal antibodies conjugated to colloidal gold to demonstrate the presence of arbovirus antigen in mosquito pools. VecTest assays that detect and separately identify West Nile virus (WN) and St. Louis encephalitis virus (SLE) on the same dipstick (VecTest WNV/SLE assay) have been evaluated in the laboratory and field (Nasci et al. 2002, Ryan et al. 2003), and are similar in specificity and sensitivity to antigen-capture enzyme-linked immunosorbent assays (AC-ELISAs) for these viruses (Tsai et al. 1987, Hunt et al. 2002). The objective of this research was to determine the sensitivity and specificity of VecTest antigen assays for detecting eastern equine encephalitis virus (EEE) and western equine encephalitis virus (WEE), the primary alphaviruses of public health importance in the United States.

The tests were provided by the manufacturer as the VecTest WNV/SLE/EEE assay and the VecTest WNV/SLE/WEE assay (lot number 030102), incorporating WN and SLE tests on the same strip as the EEE or WEE tests. Because the sensitivity and specificity of the WN and SLE assays have been evaluated previously (Ryan et al. 2003), they were not included in this test other than to verify that they were present and functional on the dipstick. The monoclonal antibodies (MAbs) used in the EEE and WEE VecTest assays are well characterized and have been described previously. Monoclonal antibodies 1B5C-3 and 1A4B-6 are used in the EEE virus detection system; 1B5C-3 is North American EEE specific and 1A4B-6 is alphavirus group reactive (Roehrig et al. 1990, Brown et al. 2001). Monoclonal antibodies 2B1C-6 and 2A3D-5 are used in the WEE detection system; 2B1C-6 is WEE specific and 2A3D-5 is WEE subcomplex reactive (Hunt and Roehrig 1985). Although 1A4B-6 and 2A3D-5 react with several alphaviruses, as paired with type-specific MAbs in the VecTest assays the tests will only produce positive results with the viruses detected by the more specific MAbs of the pair. Therefore, neither the WEE or EEE VecTest assay will detect Highlands J virus, an alphavirus in the WEE complex that is found in the eastern United States (Karabatsos et al. 1963, Calisher et al. 1980).

According to the package instructions, mosquito pools containing up to 50 individuals are homogenized in 2.5 ml of VecTest grinding solution, centrifuged (optional step), and 250 µl of the supernatant is placed into a 1.7-ml conical tube with a single dipstick. After 15 min, the

[1] Centers for Disease Control and Prevention, Division of Vector-Borne Infectious Diseases, PO 2087, Fort Collins, CO 80522.

[2] Department of Entomology, Division of Communicable Disease and Immunology, Walter Reed Army Institute of Research, Silver Spring, MD 20910-7500.

[3] Present address: Cepheid, 120 Beth Court, Athens, GA 30605.

[4] Medical Analysis Systems, Inc., 5300 Adolfo Road, Camarillo, CA 93012.

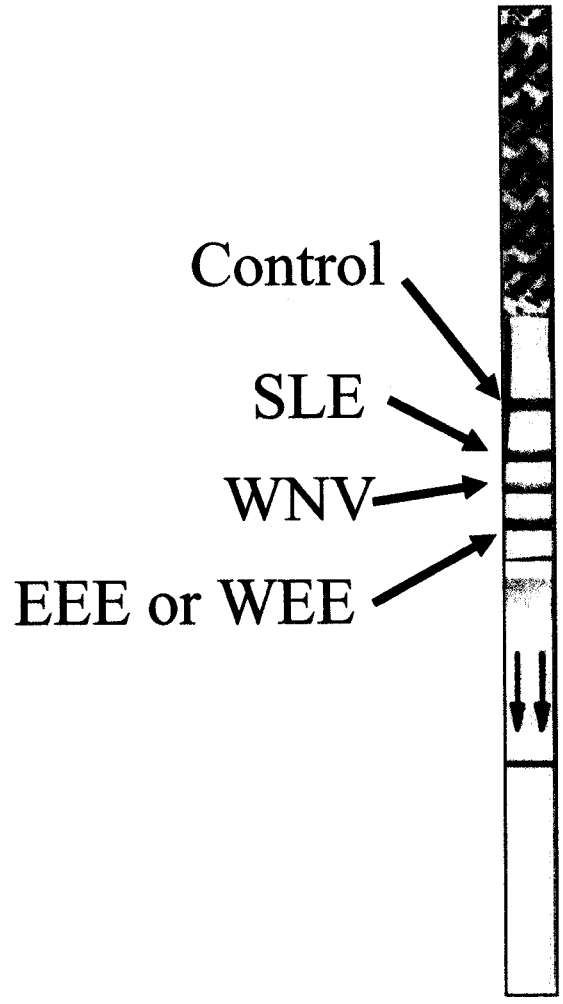

Fig. 1. The VecTest assay dipstick showing the location of the three virus detection sites and the control site on the stick.

dipstick is examined for presence of a pink band in the control region of the dipstick indicating that the test material passed through the test region and presence of a pink band in the test region. The position of the pink band in the test region is compared to a key that identifies the position of the WN, SLE, and EEE or WEE indicator spots (Fig. 1).

The virus strains used in this study (EEE [NJ-60], WEE [Flemming], SLE [TBH-28], and WN [NY-99-6922]) were derived from suckling mouse brain using standard techniques (Beaty et al. 1995). To test the sensitivity and specificity of the assays we made dilutions of EEE and WEE in VecTest grinding solution. All titers are expressed as $\log_{10}$ plaque-forming units per milliliter ($\log_{10}$ PFU/ml) as determined in a Vero cell plaque assay (Beaty et al. 1995). A 250-μl aliquot of the diluted material was transferred to a 1.7-ml conical tube. One dipstick was placed in the tube. After 15 min, the dipstick was removed from the tube, allowed to dry for several minutes, and examined for a pink band at the control spot and for any discernible pink band at the test spots. The dipsticks were also tested against high-titer solutions of SLE (5.9 $\log_{10}$ PFU/ml) and WN (6.1 $\log_{10}$ PFU/ml).

We also tested the assays against individual virus-positive *Aedes aegypti* (L.), against pools containing a single virus-positive specimen of *Ae. aegypti* and 49 negative specimens, and against 10-fold dilutions of the positive mosquito pool. Positive mosquitoes were inoculated with the viruses listed above and incubated for 7–10 days at 27°C before being used in these experiments (Rosen and Gubler 1974, Brown et al. 2001). Supernatant from negative mosquito pools was used as the medium for diluting the positive pools. Although the VecTest assays are designed to be qualitative tests indicating only presence or absence of virus, we assigned a value (1–3) to visible color bands subjectively reflecting the relative intensity of the positive reaction. This allowed us to compare strength of reaction to titer. We called a test positive only if a discernible pink to red band extended across the entire width of the strip.

Results from the VecTest WNV/SLE/EEE assay evaluation are shown in Table 1. Dilutions containing 5.3 $\log_{10}$ PFU EEE/ml produced a detectable positive band at the EEE site and produced markedly more intense bands at higher titers. A positive band also was produced by the single EEE-positive mosquito (6.4 $\log_{10}$ PFU/ml) and the EEE-positive pool (5.6 $\log_{10}$ PFU/ml), but not from the diluted pool material that contained $\leq$4.6 $\log_{10}$ PFU/ml. The EEE band did not cross with WEE at 6.1 $\log_{10}$ PFU/ml. The SLE and WN produced strong positive reactions at the appropriate locations on the strip, and did not show any evidence of cross-reactions or of nonspecific binding at the EEE site.

Results from the VecTest WNV/SLE/WEE evaluation are shown in Table 2. The sample containing 4.7 $\log_{10}$ PFU WEE/ml produced a detectable positive band at the WEE site and more intense bands were observed at higher titers. A positive band also was observed from the single WEE-positive mosquito (5.7 $\log_{10}$ PFU/ml) and the WEE-positive pool (5.2 $\log_{10}$ PFU/ml), but not from the pool dilutions that contained $\leq$4.2 $\log_{10}$ PFU/ml. The WEE band did not cross with EEE at 7.3 $\log_{10}$ PFU/ml. The SLE and WN produced strong positive reactions at the appropriate locations on the strip, and did not show any evidence of cross-reactions or of nonspecific binding at the WEE site.

Optimally, procedures to detect viruses in mosquito-based arbovirus surveillance programs should be sensitive, specific, rapid, and economical.

Table 1. Sensitivity of VecTest eastern equine encephalitis virus (EEE) assay.

Virus[1]	Titer[2]	VecTest assay results[3]		
		EEE band	WN band	SLE band
EEE	8.3	3	—	—
	7.3	2	—	—
	6.9	2	—	—
	6.6	2	—	—
	6.3	1	—	—
	6.0	1	—	—
	5.9	1	—	—
	5.6	1	—	—
	5.3	1	—	—
	5.0	—	—	—
	4.3	—	—	—
	3.3	—	—	—
WEE	6.1	—		—
SLE	5.9	—		3
WN	6.1	—	3	—
EEE + mosquito	6.4	1	—	—
Negative mosquito	—	—	—	—
EEE + pool, undiluted	5.6	1	—	—
EEE + pool^{-1}	4.6	—	—	—
EEE + pool^{-2}	3.6	—	—	—
EEE + pool^{-3}	2.6	—	—	—
EEE + pool^{-4}	1.6	—	—	—
Negative pool	—	—		—

[1] WEE, western equine encephalitis virus; SLE, St. Louis encephalitis virus; WN, West Nile virus.
[2] $\log_{10}$ plaque forming unit/ml.
[3] Discernible bands were assigned a subjective score of 1–3 (1 = lowest intensity; 3 = highest intensity) relative to the intensity of the color reaction.

Table 2. Sensitivity of VecTest western equine encephalitis virus (WEE) assay.

Virus[1]	Titer[2]	VecTest assay results[3]		
		WEE band	WN band	SLE band
WEE	7.1	3	—	—
	6.1	2	—	—
	5.7	2	—	—
	5.4	2	—	—
	5.1	1	—	—
	4.8	1	—	—
	4.7	1	—	—
	4.4	—	—	—
	4.1	—	—	—
	3.8	—	—	—
	3.1	—	—	—
	2.1	—	—	—
EEE	7.3	—	—	—
SLE	5.9	—	—	3
WN	6.1	—	3	—
WEE + mosquito	5.7	1	—	—
Negative mosquito	—	—	—	—
WEE + pool, undiluted	5.2	1	—	—
WEE + pool^{-1}	4.2	—	—	—
WEE + pool^{-2}	3.2	—	—	—
WEE + pool^{-3}	2.2	—	—	—
WEE + pool^{-4}	1.2	—	—	—
Negative pool	—	—	—	—

[1] WEE, western equine encephalitis virus; SLE, St. Louis encephalitis virus; WN, West Nile virus.
[2] $\log_{10}$ plaque forming unit/ml.
[3] Discernible bands were assigned a subjective score of 1–3 (1 = lowest intensity; 3 = highest intensity) relative to the intensity of the color reaction.

The concentration of virus varies among virus-positive field-collected mosquito pools (Nasci and Mitchell 1996); therefore, more sensitive tests detect a greater proportion of the positive pools present in a collection than less sensitive assays can detect (Kramer et al. 2002). Tests with a high degree of specificity eliminate the need for multiple steps to identify the target pathogen after it is detected, but reduce the potential for detecting unexpected or introduced pathogens. Short laboratory turnaround times are essential if public health protection measures are to be put in place before disease risk increases. Relatively low cost is critical because the ability of the surveillance system to detect virus, particularly when transmission rates are low, is dependent upon a large sample size (Chiang and Reeves 1962, Wyshak 1973). Of the techniques currently used in arbovirus surveillance programs—isolation and identification of infectious virus in cell culture or other living systems; recognition and amplification of virus-specific RNA by using a reverse transcriptase polymerase chain reaction procedure; or identification of virus-specific antigen by using AC-ELISA (Beaty et al. 1995, Brown et al. 2001, Kramer et al. 2002)—none is ideal. Reverse transcriptase polymerase chain reaction is rapid, specific, and sensitive, but requires expensive equipment and experienced personnel. Cell culture is sensitive but slow and costly. Although AC-ELISA procedures are not as sensitive as other techniques, their specificity, ease of use, and relatively low cost makes them attractive options and several protocols have been developed for use in surveillance programs (Hildreth and Beaty 1984, Tsai et al. 1987, Brown et al. 2001, Hunt et al. 2002). Incorporation of MAbs has increased sensitivity and standardized several aspects of AC-ELISA procedures (Brown et al. 2001), but AC-ELISA test kits are not commercially available and each laboratory that uses these procedures must prepare and optimize many of the reagents.

The VecTest assays for EEE and WEE use well-defined MAbs, are type-specific, and with sensitivities of approximately 5.3 $\log_{10}$ PFU/ml and 4.7 $\log_{10}$ PFU/ml for EEE and WEE, respectively, approximate the sensitivity of AC-ELISA procedures that require 4.0–5.0 $\log_{10}$ PFU EEE or WEE/ml (Brown et al. 2001; CDC, unpublished data). When using the VecTest assays, results may be obtained in as little as 15 min after the specimens are sorted into pools and ground, and are easy to interpret. The kits do not require special equipment, reagents, or training beyond standard microbiological and biosafety knowledge. All materials required are included in the kits and are stable at room temperature for 24 months. The current cost for the VecTest is $8.00, $10.00, and $12.00 per strip for strips with 1, 2, and 3 antigen assays per strip, respectively. These attributes, and the ability to test for multiple agents on a single dipstick, make the VecTest assays an attractive alternative to the AC-ELISA for surveillance programs without facilities to conduct more expensive or complex testing. Because the VecTest assays have not been widely used in field situations, they should be considered experimental until more extensive evaluations are conducted and a good background of experience comparing VecTest results to other assays is accumulated.

The VecTest assays were developed under Small Business Innovative Research grant, Wicking Assays for the Rapid Detection of Arthropod-borne Pathogens, DAMD 17-97-C-7020.

REFERENCES CITED

Beaty BJ, Calisher CH, Shope RS, Schimdt NJ, Emmons RW. 1995. Arboviruses. In: Lenette EH, Lenette DA, Lenette ET, eds. *Diagnostic procedures for viral, rickettsial and chlamydial infections* Washington, DC: American Public Health Association. p 189–212.

Brown TM, Mitchell CJ, Nasci RS, Smith GC, Roehrig JT. 2001. Detection of eastern equine encephalitis virus in infected mosquitoes using a monoclonal antibody based antigen capture enzyme-linked immunosorbent assay. *Am J Trop Med Hyg* 65:208–213.

Calisher CH, Shope RE, Brandt W, Casals J, Karabatsos N, Murphy FA, Tesh RB, Wiebe WB. 1980. Proposed antigenic classification of registered arboviruses. 1. Togaviridae, alphavirus. *Intervirology* 14:229.

Chiang CL, Reeves WC. 1962. Statistical estimation of virus infection rates in mosquito vector populations. *Am J Hyg* 73:377–391.

Hildreth SW, Beaty BJ. 1984. Detection of eastern equine encephalomyelitis virus and Highlands J virus antigens within mosquito pools by enzyme immunoassay (EIA). *Am J Trop Med Hyg* 33:965–972.

Hunt AR, Hall RA, Kerst AJ, Nasci RS, Savage HM, Panella NA, Gottfried KL, Burkhalter KL, Roehrig JT. 2002. Detection of West Nile virus antigen in mosquitoes and avian tissues by a monoclonal antibody-based capture enzyme immunoassay. *J Clin Microbiol* 40:2023–2030.

Hunt AR, Roehrig JT. 1985. Biochemical and biological characteristics of epitopes on the E1 glycoprotein of western equine encephalitis virus. *Virology* 142:334–336.

Karabatsos N, Bourke ATC, Henderson JR. 1963. Antigenic variation among strains of western equine encephalomyelitis virus. *Am J Trop Med Hyg* 12:408.

Kramer LD, Wolfe TM, Green EN, Chiles RW, Fallah H, Fang Y, Reisen WK. 2002. Detection of encephalitis viruses in mosquitoes (Diptera: Culicidae) and avian tissues. *J Med Entomol* 39:312–323.

Nasci RS, Gottfried KL, Burkhalter KL, Kulasekera VL, Lambert AJ, Lanciotti RS, Hunt AR, Ryan JR. 2002. Comparison of Vero cell plaque assay, TaqMan reverse transcriptase RNA assay, and VecTest antigen assay for detection of West Nile virus in field-collected mosquitoes. *J Am Mosq Control Assoc* 18:294–300.

Nasci RS, Mitchell CJ. 1996. Arbovirus titer variation in field-collected mosquitoes. *J Am Mosq Control Assoc* 12:167–171.

Roehrig JT, Hunt AR, Chang GJ, Sheik B, Bolin RA, Tsai TF, Trent DW. 1990. Identification of monoclonal antibodies capable of differentiating antigenic varieties of eastern equine encephalitis viruses. *Am J Trop Med Hyg* 42:394–398.

Rosen L, Gubler D. 1974. The use of mosquitoes to detect and propagate dengue viruses. *Am J Trop Med Hyg* 23: 1153–1160.

Ryan J, Davé K, Emmerich E, Fernández B, Turell M, Johnson AJ, Gottfried KL, Burkhalter KL, Kerst AJ, Hunt AR, Nasci RS. 2003. Wicking assays for the rapid detection of West Nile and St. Louis encephalitis viral antigens in mosquitoes. *J Med Entomol* 40:95–99.

Tsai TF, Bolin RA, Montoya M, Bailey RE, Francy DB, Mozan M, Roehrig JT. 1987. Detection of St. Louis encephalitis virus antigen in mosquitoes by capture enzyme immunoassay. *J Clin Microbiol* 25:370–376.

Wyshak G. 1973. Determination of sample size. *Am J Epidemiol* 97:1–3.