Assays to Detect West Nile Virus in Dead Birds

Ward B. Stone,* Joseph E. Therrien,* Robert Benson,* Laura Kramer,† Elizabeth B. Kauffman,† Millicent Eidson,† and Scott Campbell‡

Using oral swab samples to detect West Nile virus in dead birds, we compared the Rapid Analyte Measurement Platform (RAMP) assay with VecTest and real-time reverse-transcriptase–polymerase chain reaction. The sensitivities of RAMP and VecTest for testing corvid species were 91.0% and 82.1%, respectively.

Since the discovery of West Nile virus (WNV) in New York in 1999, an integral part of monitoring has been testing dead bird tissue by using real-time and standard reverse-transcriptase–polymerase chain reaction (RT-PCR) (1–3). The detection limit for WNV by both methods is as low as 0.08 PFU (1.9 log10 PFU/mL), which indicates that RT-PCR is more sensitive than cell culture and more accurately indicates infection, since RNA is more stable than infectious virus in tissues (3). Recent studies have assessed potential time- and cost-saving alternatives such as VecTest (Medical Analysis Systems Camarillo, CA, USA) (4–9). Although studies have found that VecTest, with a detection limit in mosquitoes of 5.17 log10 PFU/mL (10), is less sensitive than RT-PCR for detecting WNV, test sensitivity was generally high when testing swab samples from corvid species (4,6,8,9) and certain noncorvid species such as House Sparrows (Passer domesticus) (4) and North American Owls (family Strigidae) (7). Disadvantages were occasional atypical results, including false-positives (4).

In this study we evaluated another alternative for WNV detection, the Rapid Analyte Measurement Platform (RAMP, Response Biomedical Corp, Burnaby, British Columbia, Canada). Limited studies conducted at the Centers for Disease Control and Prevention and the Canadian National Microbiology Laboratory indicated that the RAMP WNV test, with detection limits in mosquitoes as low as 3.17 log10 PFU/mL, was more sensitive than VecTest (10). Both tests incorporate immunochromatographic test strips by using labeled antibodies to detect antigen in samples. VecTest uses antibodies bound to gold sol particle labels, while the RAMP test uses antibodies bound to fluorescently labeled latex particles. Development of a visible reddish-purple line in both the test and control zones on the VecTest strip indicates a positive result. The RAMP test strip, enclosed within a cartridge, is inserted into a reader that calculates the ratio between the fluorescence emitted at the test and control zones and displays the results as RAMP units. Values above a background threshold are recorded as positive.

This study compared WNV results from the RAMP and VecTest on oral swab samples from dead birds, with RT-PCR on brain tissue as the standard. Brain swab samples were also tested as an alternate antigen source in the RAMP and VecTest.

The Study

Birds included in this study were received from mid-May to late November 2004 and from mid-February through May 2005 from counties in New York State. Oral swab samples for the RAMP and VecTest were collected with 2 sterile, polyester fiber-tipped plastic applicators held together and moved around the oral cavity and proximal esophagus. One swab sample was twirled in 1.0 mL of VecTest buffer solution in a 5-mL plastic tube. The second swab sample was either twirled in 1.0 mL of RAMP buffer solution in a separate 5-mL plastic tube or placed in an empty 5-mL plastic tube, capped, and frozen at –20ºC for later testing. RAMP tests were run the same day on fresh material or later on frozen samples. Before being tested, all frozen samples were thawed at room temperature; swabs not previously mixed in solution and swabs from thawed carcasses were then mixed in RAMP buffer solution. Samples were taken from the brains of a subset of corvid species by swabbing cerebral parenchyma and processing as for oral samples. The RAMP and VecTest were run according to manufacturers’ directions in a class II biosafety cabinet at the New York State Department of Environmental Conservation’s Wildlife Pathology Unit. RAMP test values ≥50, calculated by the RAMP reader, were recorded as positive. Differences in test performance were assessed by chi-square analysis. Data are expressed as a percentage in text and tables only when n is ≥10.

Brain samples for RT-PCR were taken at necropsy and frozen at –20ºC. Brain tissue was analyzed at the Arbovirus Laboratory, Wadsworth Center, New York State Department of Health, as described previously (2,3). RT-PCR was repeated on 54 birds for which results from RAMP, VecTest, or both, contrasted with RT-PCR results. Retests of 6 birds yielded different results from the original tests. Three of these were initially positive and retested negative; the original values were low, which indicated infectivity was focal and undetected on a different sample,
and the level was below RAMP and VecTest limits of
detection. Three originally negative samples retested posi-
tive; 2 were highly positive, which indicated a technical
error, and 1 kidney tissue sample was positive, although
results of a retest with brain tissue were negative.

In this study, oral samples from 679 birds were tested;
193 (28.4%) were WNV-positive by RT-PCR. RAMP sen-
sitivity was 80.8%, compared to 71.0% for VecTest
(Table 1). For corvid species (n = 156), RAMP sensitivity
(91.0%) was significantly greater than that of VecTest
(82.1%) (p < 0.025). With smaller sample sizes at the
species level, sensitivity between RAMP and VecTest did
not differ significantly (p > 0.05) for 128 American Crows
(Corvus brachyrhynchos) (91.4% and 84.4%, respectively)
and 27 Blue Jays (Cyanocitta cristata) (88.9% and 70.4%,
respectively) tested, nor for interspecies differences within
each test. The detection thresholds of these tests, coupled
with viral titers of specimens, may explain these different
results.

RAMP confirmed more Common Grackles (Quiscalus
quiscula) (3/3) and House Sparrows (5/6) as positive than
did VecTest (2/3 and 3/6, respectively). With the exception
of a few species, both tests performed poorly overall on
small sample sizes of other noncorvid species.

To determine if RAMP results were affected by freez-
ing the sample, samples from 13 corvids (10 positive,
3 negative) were retested by using swabs taken from
frozen carcasses. Six initially were tested with fresh swabs
and 7 with frozen swabs; all retests yielded results similar
to initial results. The same results for fresh versus frozen
samples were obtained with VecTest (4).

VecTest specificity with oral swabs was excellent in
correctly identifying all 486 RT-PCR–negative birds,
returning no false-positive results (Table 2). RAMP had
high specificity for American Crows (98.5%), Blue Jays
(90.9%), and noncorvid species (98.9%).

Brain swab samples from 39 corvids were tested; 27
(69.2%) were RT-PCR–positive. Both RAMP and VecTest
performed well, with sensitivities of 92.6% and 88.9%,
respectively, and no false-positive results.

Conclusions

Although RAMP was more sensitive than VecTest, both
appear adequate for WNV surveillance in dead corvids.
RAMP also performed well with oral swabs from
Common Grackles and House Sparrows, although sample
sizes were small. These findings are similar to previous
results for VecTest, which also tested well with House
Finches (Carpodacus mexicanus), Northern Cardinals
(Cardinalis cardinalis), and American Kestrels (Falco
sparverius) (4). As in the previous study, both tests did
poorly in RT-PCR–positive raptors.

Table 1. Oral swab RAMP and VecTest sensitivity for real-time RT-PCR–positive birds. New York, 2004–2005*

| Species (presented in taxonomic order) | N† | RAMP | VecTest |
|----------------------------------------|----|------|---------|
| Pelicaniformes                          |    |      |         |
| Double Crested Cormorant (Phalacrocorax auritus) | 1  | 0    | 0       |
| Falconiformes                           |    |      |         |
| Cooper’s Hawk (Accipiter cooperi)       | 4  | 0    | 0       |
| Red-tailed Hawk (Buteo jamaicensis)     | 2  | 0    | 0       |
| American Kestrel (Falco sparverius)     | 1  | 1    | 1       |
| Charadriiformes                         |    |      |         |
| Ring-billed Gull (Larus delawarensis)   | 1  | 1    | 0       |
| Strigiformes                            |    |      |         |
| Great Horned Owl (Bubo virginianus)     | 3  | 1    | 0       |
| Passeriformes                           |    |      |         |
| Eastern Kingbird (Tyrannus tyrannus)    | 1  | 0    | 0       |
| Red-eyed Vireo (Vireo olivaceus)        | 1  | 0    | 0       |
| Blue Jay (Cyanocitta cristata)          | 27 | 24 (88.9) | 19 (70.4) |
| American Crow (Corvus brachyrhynchos)  | 128| 117 (91.4) | 108 (84.4) |
| Fish Crow (Corvus ossifragus)           | 1  | 1    | 1       |
| American Robin (Turdus migratorius)     | 7  | 1    | 1       |
| Gray Catbird (Dumetella carolinensis)   | 1  | 0    | 0       |
| Northern Mockingbird (Mimus polyglottos) | 2 | 1    | 1       |
| Cedar Waxwing (Bombycilla cedrorum)     | 1  | 1    | 1       |
| Northern Cardinal (Cardinalis cardinalis) | 1 | 0    | 0       |
| Common Grackle (Quiscalus quiscula)     | 3  | 3    | 2       |
| House Finch (Carpodacus mexicanus)      | 2  | 0    | 0       |
| House Sparrow (Passer domesticus)       | 6  | 5    | 3       |
| Total all species                      | 193| 156 (80.8) | 137 (71.0) |

* RAMP, Rapid Analyte Measurement Platform; RT-PCR, reverse transcription–polymerase chain reaction.
†No. of birds real-time RT-PCR–positive.
In the previous New York study, VecTests successfully tested brain, kidney, blood, feather pulp, and cloacal samples from corvids and House Sparrows (4). In the current study, RAMP and VecTest worked well with brain as the antigen source. Brain swab samples may be the preferred antigen source when the oral cavity is compromised. Further testing of alternative swab samples is warranted and may identify a superior antigen source; however, testing internal organs may pose greater risks and may not be applicable in field work and nonlaboratory-based surveillance. In addition, further testing, including immunohistochemical tests, on noncorvids should be conducted to accurately assess these tests and identify the distribution of WNV in the oral cavity and internal tissues.

In this study, VecTest produced no false-positive results. Although its specificity was high, RAMP produced 8 false-positive results (range 50.9–147.6). Four of these were near the >50 positive indicator level and may have been due to other sources of fluorescence. The remaining 4 false-positives (3 American Crows and 1 Blue Jay), with scores from 74.4 to 147.6, came from birds with oral cavities compromised by blood or fly eggs, which may have biased results.

VecTest results are easily distinguished when a true WNV-positive reaction occurs, but the reddish-purple line may appear faint or thin in other cases and may be subject to interpretation (4). RAMP quantitative results eliminate subjective interpretation, which helps assure replication but limits confidence in lower RAMP-positive scores.

The RAMP system requires an initial purchase of an electronic reader (~US $3,500), and materials cost $13–$15 per test; VecTest costs $8 per test. If large numbers of specimens are tested, the cost of the RAMP reader per test is minimal. The RAMP test requires a minimum of 1.5 h to run because of the required cartridge drying time; VecTest takes 15–30 min to run after the test strip is placed in the sample solution.

In conclusion, both RAMP and VecTest are useful alternatives to RT-PCR for WNV surveillance in dead corvids and some passerine species when immediate turn-around of large numbers of specimens is valuable. Testing with RAMP is advantageous because of its increased sensitivity; however, follow-up testing with RT-PCR is recommended for low RAMP-positive results near the positive indicator level. Using both tests in a system in which initial testing is conducted with VecTest may also be useful; RAMP could be reserved for high-priority cases in which VecTest results are negative. RT-PCR should still be used to confirm initial viral activity in a new period and area and for research requiring more definitive results.

Acknowledgments
We thank Kevin Hynes, Joe Okoniewski, and Darci Dougherty for their technical assistance; Dave Galinski for bird necropsy, swab and tissue collection, performance of RAMP, and VecTest; Susan Jones and Mary Franke for real-time RT-PCR assays; county health departments, New York City Department of Health and Mental Hygiene, Yoichiro Hagiwara, Richard Chipman, Richard A. Watt and other animal control officers, wildlife rehabilitators, and the concerned public for coordinating and participating in dead bird reporting and submission.

The New York State Department of Environmental Conservation and the New York State Department of Health jointly supported this research. Work on this study was partially supported by the New York State Department of Environmental Conservation and the New York State Department of Health.
supported by federal funds from the Centers for Disease Control and Prevention under cooperative agreement numbers U50/CCU223671 and U90/CCU216988.

Dr Stone has been the wildlife pathologist for the New York State Department of Environmental Conservation for >36 years. He also is an adjunct professor at the State University of New York College at Cobleskill and the College of St. Rose. Dr Stone’s main research interests are in infectious and parasitic diseases, toxicology, and forensic pathology of wildlife.

References

1. Lanciotti RS. Molecular amplification assays for the detection of flaviviruses. Adv Virus Res. 2003;61:67–99.
2. Kauffman EB, Jones SA, Dupuis AP, Ngo KA, Bernard KA, Kramer LD. Virus detection protocols for West Nile virus in vertebrate and mosquito specimens. J Clin Microbiol. 2003;41:3661–7.
3. Shi P-Y, Kauffman EB, Ren P, Felton A, Tai JH, Dupuis II AP, et al. High-throughput detection of West Nile virus RNA. J Clin Microbiol. 2001:39:1264–71.
4. Stone WB, Okoniewski JC, Therrien JE, Kramer LD, Kaufman EB, Eidson M. VecTest as diagnostic and surveillance tool for West Nile virus in dead birds. Emerg Infect Dis. 2004;10:2175–81.
5. Siirin M, Sargent C, Langer RC, Parsons R, Vanlandingham DL, Higgs S, et al. Comparative sensitivity of the VecTest antigen-capture assay, reverse transcriptase-PCR, and cell culture for detection of West Nile virus in dead birds. Vector-borne and Zoonotic Diseases. 2004;4:204–9.
6. Henson G, Hicock P. Rapid detection of West Nile virus in bird using the VecTest WNV antigen assay. Clin Lab Sci. 2004;17:218–20.
7. Gancz AJ, Campbell DG, Barker IK, Lindsay R, Hunter B. Detecting West Nile virus in owls and raptors by an antigen-capture assay. Emerg Infect Dis. 2004;10:2204–6.
8. Lindsay R, Barker I, Nayar G, Drebott M, Calvin S, Scammell C, et al. Rapid antigen-capture assay to detect West Nile virus in dead corvids. Emerg Infect Dis. 2003;9:1406–10.
9. Yaremnych SA, Warner RE, Van de Wyngaerde MT, Ringia AM, Lampman R, Novak RJ. West Nile virus detection in American Crows. Emerg Infect Dis. 2003;9:1319–21.
10. Burkhalter KL, Lindsay R, Anderson R, DiBernardo A, White H, Drebott M, et al. undated. Evaluation of commercial assays for detecting West Nile virus antigen. Burnaby, British Columbia, Canada: Response Biomedical Corporation.

Address for correspondence: Ward B. Stone, NYSDEC – Wildlife Pathology Unit, 108 Game Farm Rd, Delmar, NY 12054, USA; fax: 518-478-3035; email: wbstone@gw.dec.state.ny.us