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Expanded Molecular Testing on Patients with Suspected West Nile Virus Disease

Nicole P. Lindsey[1], **Sharon L. Messenger**[2], **Jill K. Hacker**[2], **Maria L. Salas**[2], **Christine Scott-Waldron**[3], **Danielle Haydel**[3], **Errin Rider**[3], **Sean Simonson**[3], **Catherine M. Brown**[4], **Pinal Patel**[4], **Sandra C. Smole**[4], **David F. Neitzel**[5], **Elizabeth K. Schiffman**[5], **Jennifer Palm**[5], **Anna K. Strain**[5], **Sara M. Vetter**[5], **Brian Nefzger**[5], **Marc Fischer**[1], **Ingrid B. Rabe**[1]

[1]Arboviral Diseases Branch, Centers for Disease Control and Prevention, Fort Collins, Colorado

[2]Viral and Rickettsial Disease Laboratory, California Department of Public Health, Richmond, California

[3]Louisiana Office of Public Health, Baton Rouge, Louisiana

[4]Massachusetts Department of Public Health, Jamaica Plain, Massachusetts

[5]Minnesota Department of Health, Saint Paul, Minnesota

Abstract

Most diagnostic testing for West Nile virus (WNV) disease is accomplished using serologic testing, which is subject to cross-reactivity, may require cumbersome confirmatory testing, and may fail to detect infection in specimens collected early in the course of illness. The objective of this project was to determine whether a combination of molecular and serologic testing would increase detection of WNV disease cases in acute serum samples. A total of 380 serum specimens collected ≤7 days after onset of symptoms and submitted to four state public health laboratories for WNV diagnostic testing in 2014 and 2015 were tested. WNV immunoglobulin M (IgM) antibody and RT-PCR tests were performed on specimens collected ≤3 days after symptom onset. WNV IgM antibody testing was performed on specimens collected 4–7 days after onset and RT-PCR was performed on IgM-positive specimens. A patient was considered to have laboratory evidence of WNV infection if they had detectable WNV IgM antibodies or WNV RNA in the submitted serum specimen. Of specimens collected ≤3 days after symptom onset, 19/158 (12%) had laboratory evidence of WNV infection, including 16 positive for only WNV IgM antibodies, 1 positive for only WNV RNA, and 2 positive for both. Of specimens collected 4–7 days after onset, 21/222 (9%) were positive for WNV IgM antibodies; none had detectable WNV RNA. These findings suggest that routinely performing WNV RT-PCR on acute serum specimens submitted for WNV diagnostic testing is unlikely to identify a substantial number of additional cases beyond IgM antibody testing alone.

Address correspondence to: Nicole P. Lindsey, Arboviral Diseases Branch, Centers for Disease Control and Prevention, 3156 Rampart Road, Fort Collins, CO 80521, nplindsey@cdc.gov.

Author Disclosure Statement

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Introduction

WEST NIlE VIRUS (WNV) is the leading cause of domestically acquired arboviral disease in the United States (Petersen et al. 2013, Burakoff et al. 2018). There are no distinctive clinical features differentiating WNV from other viral causes of acute systemic febrile or neurologic disease. Rapid diagnosis of WNV infection can assist in clinical management, obviating the need for antimicrobial treatment and informing patient prognosis. Most diagnostic testing for WNV disease is accomplished using immunoglobulin M (IgM) immunoassays and, if positive, confirmation with plaque reduction neutralization testing (PRNT). However, WNV IgM antibody testing is subject to false-positive results and cross-reactivity with other flaviviruses, and PRNT is time consuming and performed by a limited number of laboratories.

Historically, viremia among patients with WNV infection has been found to be low-level and transient (Lanciotti et al. 2000, Lanciotti and Kerst 2001, Tilley et al. 2006, Barzon et al. 2013). It is generally accepted that by the time most immunocompetent patients present with clinical symptoms, WNV RNA is no longer detectable using standard diagnostic molecular assays. As a result, molecular assays usually are not performed on specimens submitted for routine diagnostic testing and, if performed, negative results cannot be used to rule out infection (Lanciotti et al. 2000, Lanciotti and Kerst 2001). However, considering progress in the development of molecular assays and their widespread availability, and reports of success in detecting WNV RNA in serum specimens collected from acutely ill patients, it is prudent to reexamine the utility of WNV RT-PCR testing (Tilley et al. 2006).

If sufficiently sensitive, molecular testing could provide a more timely diagnosis, improve the yield of diagnostic testing performed on serum specimens collected soon after illness onset, and overcome the limitations of cross-reactivity and interpretation of serologic results. Although cerebrospinal fluid is the preferred specimen for testing patients with possible neuroinvasive disease, it is not collected on most patients and remaining sample volumes after initial testing are not always sufficient for WNV RT-PCR testing. Therefore, testing conducted in this study was limited to serum specimens. We performed RT-PCR on acute serum specimens submitted for WNV diagnostic testing to determine whether additional WNV disease cases could be detected or confirmed with routine combination of molecular and serologic testing.

Materials and Methods

Serum specimens submitted for WNV diagnostic testing from July–September in 2014 and 2015 to four state public health laboratories (California, Louisiana, Massachusetts, and Minnesota) were evaluated. Data for patient age, sex, date of symptom onset, and date of specimen collection were obtained from submission paperwork. In addition, as part of routine public health case investigations, attempts were made to collect missing data for any

patients with positive test results. Acute serum specimens collected ≤7 days after onset of symptoms were included in the analysis. Specimens with insufficient volume or missing dates of collection or illness onset were excluded.

WNV IgM antibody and RT-PCR tests were performed on all specimens collected ≤3 days after symptom onset. WNV IgM antibody testing was performed on all specimens collected 4–7 days after onset and among those, RT-PCR was performed on IgM-positive specimens. RT-PCR was not performed on IgM-negative specimens collected >3 days after onset because the low likelihood of obtaining a positive result did not justify the additional cost and personnel time. A patient was considered to have laboratory evidence of WNV infection if they had detectable WNV IgM antibodies, WNV RNA, or both, in the submitted serum specimen.

Each state laboratory tested specimens from their own jurisdiction using their routine diagnostic assays and protocols. For IgM antibody testing, assays used included the CDC microsphere immunoassay (Louisiana), Focus Diagnostics WNV IgM Capture DxSelect enzyme-linked immunosorbent assay (ELISA; California), InBios West Nile DetectTM IgM Capture ELISA (Minnesota), and an in-house validated WNV IgM antibody capture ELISA (Massachusetts) (Martin et al. 2000, Johnson et al. 2005, Focus Diagnostics 2015, Inbios International, Inc. 2015). All four sites used the CDC WNV RT-PCR with site-specific variations in extraction volumes; RNA was extracted from 100 μL of serum in Louisiana, 140 μL of serum in California, and 200 μL of serum in Massachusetts, and Minnesota (Lanciotti et al. 2000, Lanciotti and Kerst 2001).

Demographic data for patients with and without laboratory evidence of WNV infection and for patients with specimens collected ≤3 days after versus 4–7 days after onset of symptoms were compared. Categorical variables were summarized using counts and proportions and compared using Fisher’s exact test; continuous variables were summarized using median and range and compared using the Wilcoxon rank-sum test. The data were analyzed using SAS version 9.4 (Cary, NC). These data were collected as part of public health surveillance and therefore did not require Institutional Review Board approval.

Results

Serum specimens from 158 patients collected ≤3 days after illness onset were tested for WNV IgM antibodies and RNA (Table 1). Of these, 19 (12%) had laboratory evidence of WNV infection, including 15 (45%) of 33 from California, 2 (2%) of 109 from Massachusetts, 1 (14%) of 7 from Minnesota, and 1 (11%) of 9 from Louisiana. Overall, 16 (84%) of 19 were positive only for WNV IgM antibodies, 1 (5%) was positive only for WNV RNA, and 2 (11%) were positive for both IgM antibodies and RNA. The three RNA-positive specimens were identified in California (n=2) and Louisiana (n=1). Of the 19 WNV disease cases identified ≤3 days after onset of symptoms, 11 (58%) were female compared to 59 (42%) of 139 patients that tested negative for WNV IgM and RNA (p=0.2). The median age of case-patients was 58 years (range 40–91 years) compared to 44 years (range 5–85 years) for patients who tested negative (p<0.01).

Serum specimens from 222 patients collected 4–7 days after illness onset were tested for WNV IgM antibodies. Of these, 21 (9%) had laboratory evidence of WNV infection, including 15 (48%) of 31 from California, 1 (5%) of 21 from Louisiana, 1 (3%) of 62 from Minnesota, and 0 (0%) of 108 from Massachusetts. None of the IgM-positive specimens were positive for WNV RNA. IgM-negative specimens collected at 4–7 days after onset were not evaluated by RT-PCR.

Overall, we identified 40 patients with laboratory evidence of WNV infection (Table 2). Of these, 37 (93%) were positive only for WNV IgM antibodies, 1 (2%) was positive only for WNV RNA, and 2 (5%) were positive for both IgM antibodies and RNA. All three of the RNA-positive case-patients were male compared to 17 (46%) of the 37 case-patients who were RNA-negative. The median age of case-patients with positive RT-PCR was 50 years (range 50–74 years) compared to 64 years (range 12–91 years) for those with negative RT-PCR testing. Finally, the three RNA-positive specimens were collected a median of 2 days after onset of symptoms (range 1–3 days) compared to 4 days after onset (range 0–7 days) for the 37 specimens that were IgM-positive but RNA-negative.

Discussion

The findings from this study suggest that routinely performing WNV RT-PCR on acute serum specimens submitted for WNV diagnostic testing is unlikely to identify a substantial number of additional cases beyond IgM antibody testing alone. Among 40 patients with evidence of WNV infection on acute serum specimens collected ≤7 days after onset of symptoms, only three (8%) specimens had detectable RNA by RT-PCR. Of those three, all were collected within 3 days of illness onset, and two were positive for IgM antibodies.

Molecular testing was also not useful to confirm WNV IgM antibody test results. Only 2 (11%) of 18 IgM-positive specimens collected ≤3 days after onset were positive for RNA on the same acute specimen, and none of the 21 IgM-positive sera collected 4–7 days after onset were positive by RT-PCR. These data further support the idea that specimens with detectable IgM antibodies are not likely to have detectable RNA and suggest that RT-PCR would have limited utility confirming serologic results or reducing the need for neutralizing antibody testing, even in early acute specimens.

Molecular testing implemented during this surveillance project only identified one additional WNV infection among seronegative patients. In contrast, a previous study performed in Canada in 2003 found that among 212 laboratory-confirmed WNV disease cases evaluated by both serology and nucleic acid amplification testing (NAT), 65 (31%) were positive by both NAT and IgM antibody testing and 25 (12%) were positive by NAT alone (Tilley et al. 2006). In that study, RNA was extracted from 1.0mL of plasma, a substantially larger specimen volume and different specimen type than was used in our project. In addition, the study included a large proportion (83%) of patients with generalized febrile illness, as opposed to neuroinvasive disease. We do not have clinical syndrome for the WNV cases identified in our project; however, patients with neurologic illness may be identified later in the course of illness and be less likely to have remaining RNA detectable in blood.

Although not a primary finding of this project, we did observe substantial differences in percent positivity among the four states. These differences could be due to a variety of factors, including differences in established submission criteria and/or WNV disease prevalence (Centers for Disease Control and Prevention 2018). Additionally, the specimens included in this evaluation were limited to those for which onset dates were available. The percentage of specimens submitted with recorded onset date varies by state. Specimens with positive test results were more likely to have those dates obtained retrospectively because of routine case follow-up procedures in the respective health departments. This approach may overestimate the proportion that was IgM positive in states with high proportions of specimens missing onset date at the time of submission. It is also possible that the different extraction volumes routinely used by each state may have impacted positivity rates, but the difference is unlikely to have been significant given the relatively small differences in volumes used.

The results presented here are subject to several limitations. First, the number of confirmed WNV disease cases is relatively small, and there were limited clinical and epidemiologic data available on the specimens submitted to the public health laboratories, which may not be representative of all suspected WNV disease cases. WNV IgM antibodies can persist for many months and even years in some individuals, so IgM positives may not always identify acute infections. Because convalescent specimens were not collected, we do not know the true number of WNV infections among the sample and initial negative results may merely reflect specimen collection before development of a detectable antibody response. Unfortunately, it is very difficult to obtain convalescent sera, particularly when patients have recovered; this is a challenge to serologic testing for WNV that is unlikely to be overcome. Additionally, the samples positive by IgM immunoassay were not all subjected to confirmatory PRNT. If a substantial proportion of those IgM results were false-positives and not true infections, the statistical power inherent in this sample set to evaluate the usefulness of RT-PCR would be reduced.

Despite the lack of demonstrated utility in this evaluation, RT-PCR may have utility in certain clinical settings. For example, molecular testing may prove useful in immunocompromised patients, when antibody development is delayed or absent (Rabe et al. 2013). Furthermore, increasing the RNA extraction volume, using more sensitive molecular assays (*e.g.*, transcription-mediated amplification assays used for blood donor screening), or testing different sample types (*e.g.*, plasma, whole blood) could possibly increase the sensitivity of such testing and the number of identified cases. However, such changes may not be feasible for routine diagnostic testing, which is why standard assays, protocols, and specimen type and volume were used in this evaluation.

Our findings support the continued use of IgM immunoassay and neutralizing antibody testing for routine diagnosis of WNV infection, without addition of molecular testing. Should technological advances in laboratories allow for the development of more sensitive molecular assays, with acceptable specificity, subsequent reevaluation of testing algorithms would be warranted. Health care providers should consult with state and local health departments to determine when additional testing modalities, including molecular testing, may be indicated for particular patients.

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Table 1.

Patients With and Without Laboratory Evidence of West Nile Virus Infection in Serum Specimens Collected ≤ 3 Days After Onset of Illness

	With laboratory evidence of WNV infection (n = 19)	***Without laboratory evidence of WNV infection (n = 139)***
	n *(%)*	**n *(%)***
Median age, years (range)	58 (40–91)	44 (5–85)
Female	11 (58)	59 (42)
Positive WNV test		
IgM antibodies only	16 (84)	—
RNA only	1 (5)	—
Both IgM and RNA	2 (11)	—
Location		
California	15 (79)	18 (13)
Massachusetts	2 (11)	107 (77)
Minnesota	1 (5)	6 (4)
Louisiana	1 (5)	8 (6)

A patient was considered to have laboratory evidence of WNV infection if they had detectable WNV IgM antibodies, WNV RNA, or both, in the submitted serum specimen.

IgM, immunoglobulin M; WNV, West Nile virus.

Table 2.

West Nile Virus Disease Cases by RT-PCR Test Results

	***RNA-positive*[a] (n = *3*)**	***RNA-negative*[b] (n = *37*)**
	n *(%)*	**n *(%)***
Median days after onset (range)	2 (1–3)	4 (0–7)
Median age, years (range)	50 (50–74)	64 (12–91)
Female	0 (0)	20 (54)
Location		
California	2 (66)	28 (76)
Massachusetts	0 (0)	2 (5)
Minnesota	0 (0)	2 (5)
Louisiana	1 (33)	5 (14)

[a] Two of the three WNV RNA-positive specimens also were positive for WNV IgM antibodies.

[b] All 37 WNV RNA-negative specimens were positive for WNV IgM antibodies.