Performance of a Commercial Immunoglobulin M Antibody Capture Assay Using Analyte-Specific Reagents To Screen for Interfering Factors during a West Nile Virus Epidemic Season in Nebraska[∇]

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In 2003, the Nebraska Public Health Laboratory tested more than 10,371 serum and 516 cerebral spinal fluid specimens. Results showed that without performing the interfering factors screen for specimens in the low positive index value range of >1.1 to ≤3.5, a false positivity rate of 6.5% would have been realized.

Human West Nile virus (WNV)-caused infections in the United States were first recognized in New York during the summer of 1999 (6). In 2003, the Nebraska Public Health Laboratory (NPHL) commenced WNV-specific immunoglobulin M (IgM) antibody testing on serum and cerebral spinal fluid (CSF) specimens. The reagent pack of the Focus Diagnostics flavivirus-specific IgM antibody capture enzyme-linked immunosorbent assay (MAC-ELISA), consisting of analyte-specific reagents (ASR), was used. Early in the WNV testing season, Nebraska state epidemiologists randomly selected 10 positive specimens, from seven patients, to send to the Centers for Disease Control and Prevention (CDC) for confirmation. The CDC MAC-ELISA and/or the plaque reduction neutralization test (PRNT) found that 4 of 10 (40%) specimens submitted, within the positive index value range of >1.1 to ≤3.5, were not confirmed and were reported as being negative or equivocal or needing a second draw. Because of the problems of interfering factors (IF) inherent in MAC-ELISA coupled with the CDC's discordant results, the NPHL decided to reflex test specimens within the "low positive" index value range of >1.1 to ≤3.5 using an interfering factors screen (IFS) which allowed for the subtraction of background absorbance. This IFS would detect possible IF that may consist of either natural antibodies or autoantibodies, including heterophile antibodies (HA), Forrsman antibodies, rheumatoid factor (RF), and other interfering substances (3, 4, 5, 7, 8, 11, 13, 16).

Between 1 August and 31 October 2003, automated testing of 10,887 specimens, consisting of 10,371 serum and 516 CSF specimens, was performed on a MAGO Plus automated enzyme immunoassay analyzer (Diamedix, Miami, FL). Serum and CSF specimens were tested at dilutions of 1:100 and 1:2, respectively. A total of 2,282 (21%) of the 10,887 specimens were positive for WNV-specific IgM by the Focus Diagnostics MAC-ELISA. The IFS was run manually on 794 of 2,282

(35%) WNV-specific IgM-positive specimens with index values ranging from >1.1 to ≤3.5. The 794 specimens tested consisted of 770 serum and 24 CSF specimens. A Tecan 96 PW microtiter plate washer (Research Triangle Park, NC) was used for the washing steps. Optical density readings at 450 nm were taken on a BioTek 800 UV microtiter plate reader (Winooski, VT). A total of 52 of the 794 (6.5%) positive specimens were found to contain IF at levels that would change qualitative test results from "positive" to "indeterminate" once the background optical density was subtracted. These samples were then categorized as "indeterminate" due to the high levels of IF present.

At the conclusion of the WNV testing season, retrospective IFS was conducted on 457 serum and 32 CSF specimens to ascertain the distribution range of naturally occurring IF. These specimens consisted of 126 positive (index values of >3.5), 81 equivocal, and 282 negative specimens. Of the 126 positive serum specimens tested, none were found to have IF at a level that would change their qualitative result. IFS results for the 81 serum specimens within the equivocal range were as follows: 8 of 81 (10%) were negative, 13 of 81 (16%) remained equivocal, 32 of 81 (40%) became positive, and 28 of 81 (35%) were indeterminate. Results showed that 64 of 282 (23%) of the negative specimens had IF present. However, qualitative test results would not be changed for either the equivocal or the negative specimens (14). The composite results of the IFS performed on the 1,283 serum and CSF specimens tested are shown in Table 1. Results indicate that the number of positive specimens decreased from 920 to 900 (2.1%), negative specimens decreased from 282 to 226 (23%), equivocal specimens decreased from 81 to 8 (90%), and 144 (11.2%) specimens contained IF. Although the 64 and 28 WNV-specific IgM-negative and equivocal samples, respectively, were found to have IF, interpretation would have remained negative or equivocal regardless of the presence of IF found in subsequent testing.

Of the total 1,283 samples that underwent the IFS, 80 (6.2%) specimens in the positive and equivocal ranges were determined to contain IF. These serum specimens, when sample volumes permitted, underwent testing for HA using the

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TABLE 1. Analysis of the use of the interfering factor screen for MAC-ELISA specimens

Index value range	Qualitative interpretation	No. of ASR tested	No. of ASR with indicated result after IFS			
			Negative	Equivocal	Positive	Indeterminate[d]
0–<0.9	Negative	282[a]	218	0	0	64[b]
>0.9–≤1.1	Equivocal	81[a]	8	13	32	28[c]
>1.1–<3.5	Low positive	794	0	0	742	52
>3.5	High positive	126[a]	0	0	126	0

[a] Specimens tested in retrospective study.
[b] Qualitative result of "negative" would have remained unchanged if package insert had been followed.
[c] Qualitative result of "equivocal" would have remained unchanged if package insert had been followed.
[d] Qualitative result not reportable due to high levels of IF.

Color Slide II mononucleosis test (Monospot; Seradyn, Indianapolis, IN) and testing for RF using the N Latex RF assay on the BN Prospec nephelometer (Dade Behring, Inc., Deerfield, IL) to try to further identify and characterize the nature of the IF present. However, testing showed that only 8 of 65 (12%) specimens tested were positive for RF and 6 of 42 (14%) specimens tested were positive for HA. Results are shown in Table 2.

The PRNT was performed by the Viral and Rickettsial Disease Laboratory, California Department of Health Services, Richmond, CA (2). Briefly, equal volumes of a standard dose (100 PFU per 100 μl) of WNV were mixed with serum specimens that had been diluted at 1:20, 1:40, and 1:80. Six of 78 (8%) specimens tested were positive for neutralizing WNV antibodies by the PRNT screen. Although these specimens were found to contain IF, none of the six PRNT-positive specimens were positive for either RF or HA. Results are shown in Table 2.

Statistical calculations were performed using commercial software (SPSS, Inc., Chicago, IL) (1). Combined analysis of the testing results of the serum and CSF specimens determined the kappa value to be 0.83 (95% confidence interval, 0.80 to 0.86).

Our testing showed that the results obtained by the Focus Diagnostics MAC-ELISA compared to those obtained by the IFS showed minimal differences, as demonstrated by a kappa value of 0.83. We also found that a false positivity rate of 6.5% (52 out of 794) would have been realized in the low positives, within the index value range of >1.1 to ≤3.5, during the 2003 testing season due to the presence of IF in patient specimens. Only a small percentage of IF could be attributed to either RF (12%) or heterophile antibodies (14%), and 8% of the serum specimens containing IF were also found to have neutralizing antibody for WNV.

Our prospective and retrospective study data agreed well

with the testing results obtained at the Focus Diagnostics reference laboratory and other clinical laboratories (9, 10, 12, 15). If one considers the fact that none of the "high positives" (index value of >3.5) tested in the retrospective study were found to have IF, only an overall 2.3% false positivity rate could have been projected.

The NPHL has continued use of the IFS for reflex testing of WNV-specific IgM-positive specimens since the 2003 season, and the IFS is also included in the standard Focus WNV IgM ELISA kit. Specimens found to have IF and hence causing the qualitative results to change from positive to equivocal or negative are reported to be negative.

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TABLE 2. Analysis of IF-positive specimens

Test performed	No. (%) of specimens[a]	
	Tested	Positive
RF	65	8 (12)
HA	42	6 (14)
PRNT	78	6 (8)

[a] No specimens tested were positive by both the RF and HA tests. No specimens positive by the PRNT were found to contain either RF or HA by the test performed.

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