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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), Forssman 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
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.

This study was made possible through a coordinated team effort involving staff at the University of Nebraska Medical Center, Regional Pathology Associates Client Services, the Nebraska Medical Center Clinical Microbiology Department, and the NPHL. The NPHL thanks Focus Diagnostics for providing consultation on testing methodology and for the MAC-ELISA reagent kits used for the background subtraction assay during the 2003 season.

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