NPHL West Nile Virus Testing Methodology

by Tony Sambol, Assistant Director, NPHL
and Beth Schweitzer, Microbiology Specialist, NPHL

Due to the lack of an FDA approved assay, very few laboratories in the United States performed West Nile Virus Nile (WNV) antibody tests during the 2003 outbreak. Now that an approved assay is available and the virus has reached the Midwest some local laboratories are considering adding the test to their menu. To assist in this effort, the NPHL has reviewed the experience it gained last year in the testing of over 10,000 samples and the evaluation of two different test formats. One of the most important discoveries was the identification of a problem with interfering antibodies or other substances (referred to as Interfering Factors or IF) causing false positive results.

The following information is intended to provide background for laboratories in Nebraska that are considering bringing the test in house, and for other laboratories that may need more information to provide explanations of test results for their local providers.

West Nile Virus infections in the United States were first recognized in New York State during the summer of 1999. The virus has since moved across the US, with the first human case detected in Nebraska in 2002. The NPHL began testing for human WNV IgM antibody in 2003 using non-Food and Drug Administration (FDA) approved reagents from Focus Technologies. The NPHL only performed the IgM antibody screen in 2003. These reagents were subsequently incorporated into a test kit that has now received FDA approval. However, during the evaluation of the reagents used by the NPHL, it was discovered that a number of specimens that screened positive could not be confirmed using the confirmatory test called the Plaque Reduction Neutralization Assay (PRNT). This finding led to additional investigations and the realization that most of the false positives were caused by the presence of Interfering Factors (IF) in the patient serum. IF are represented by different types of antibodies including Heterophile antibodies, Rheumatoid factor (RF), Forssman antibodies (serum sickness) as well as other factors. Heterophile antibodies were first shown to be induced in the presence of Epstein Barr Virus (EBV) and are the detection target for most Infectious Mononucleosis assays. EBV infections are common and may be present in a latent state in more than 80% of humans. A second screening method using a "normal control" preparation that did not include WNV antigen was added to identify those WNV specimens with IF with the goal of reducing the number of false positives.

The Focus Technologies Flavivirus (West Nile Virus) ELISA IgM Reagent Pack, used in both tests performed at NPHL, consisted of analyte specific reagents. The Focus Technologies reagents were used for qualitative detection of IgM antibodies in serum and cerebral spinal fluid (CSF). The test was performed on the Diamedix MAGO® Plus Automated Enzyme Immunoassay (EIA) Analyzer. The original optical density (OD) reading from the instrument of each test well was recorded and divided by the mean of the calibrator OD to generate an index value. Test result index values used in the evaluation were originally defined by Focus Technologies as $\leq$0.9, IgM negative; >0.9 to $\leq$1.1, IgM equivocal; and >1.1 IgM positive. Data analysis was performed on specimens obtained between August 1 and October 31. During that time 10,887 specimens were tested, of which 10,371 (95.3%) were sera and 516 (4.7%) were CSF. Of the specimens tested, 2,282 (21%) were determined to be above the positive cut-off level of >1.1. These specimens were separated for study on the basis of OD's and index values with special attention paid to those samples with an index value in the range of >1.1 to $\leq$3.5. The hypothesis to be tested was that this range would encompass the majority of samples with IF. This hypothesis was based on the fact that a number of specimens did not confirm positive by PRNT testing that was performed on the specimens in this range. The PRNT testing was performed at the Centers for Disease Control and Prevention's (CDC) branch at Fort Collins, CO.

Due to the discrepancies found by the CDC's PRNT testing, the IF test was performed on all screen positive samples in the range from >1.1 and $\leq$3.5 using the Focus Technologies reagents. The IF screening test was performed on 794 (34.8%) specimens. 770 (97%) of the 794 specimens tested were serum and 24 (3%) were CSF. 54 (6.8%) of the sera tested were reported as indeterminate due to the presence of IF while only one (1) of the CSFs tested was positive for IF.

The NPHL also performed IF testing on an additional 126 positive specimens that had test result values >3.5. All of these specimens gave negative results in the IF screen.

This study showed that IF was responsible for 6.9% of the indeterminate results in the "low-positive" range. Consequently, those specimens would have been reported out as false-positives if a second screening method would not have been performed. As with all screening tests, confirmatory methods are

needed such as PRNT or Heterophile antibody testing to determine the true nature of specimens.

When interpreting WNV IgM testing results that are positive but have low index values of >1.1 to ≤3.5, providers and laboratorians should be aware of false positives due to IF and that additional testing may be required when results do not correlate to the patient's condition. Additionally, antibodies detected by the WNV assay may cross react with other flaviviruses, including St. Louis Encephalitis Virus. Cross reactivity has also been observed with some Enterovirus infections.

For questions about WNV testing methodology, please call or email Tony Sambol (402-559-3032, asambol@unmc.edu) or Beth Schweitzer (402-559-6098, bschweitzer@unmc.edu).