LABORATORY SURVEILLANCE FOR WEST NILE VIRUS AND ST. LOUIS ENCEPHALITIS VIRUS IN MARYLAND FROM 2008 TO 2009

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BACKGROUND

- West Nile Virus (WNV)
  - An arthropod-borne virus (arbovirus) in the family *Flaviviridae.*
  - The leading cause of arboviral encephalitis in the United States.
  - Transmitted by the *Culex* species mosquitoes, particularly *Cx. pipiens*, *Cx. tarsalis*, and *Cx. quinquefasciatus*.
  - 80% of WNV infections are asymptomatic
  - Less than 1% of infected persons develop neuroinvasive disease.
- St. Louis encephalitis virus (SLEV)
  - Also an arbovirus in the family *Flaviviridae*.
  - Transmitted by *Cx. pipiens* and *Cx. quinquefasciatus* in the east, *Cx. nigripalpus* in Florida, and *Cx. tarsalis* and members of the *Cx pipiens* complex in western states.
  - Less than 1% of SLEV infections are clinically apparent.
  - About 40% of children and young adults with SLEV disease develop mild symptoms but almost 90% of elderly persons with SLEV disease develop encephalitis.
  - The overall case-fatality ratio is 5 to 15%.

METHODS

Laboratory Criteria for Diagnosis
- Four-fold or greater virus-specific serum antibody titer, OR
- Isolation of virus from or demonstration of specific viral antigen or genomic sequences in tissue, blood, cerebrospinal fluid (CSF), or other body fluid, OR
- Elevated virus-specific immunoglobulin (IgG) antibodies in the acute or convalescent serum specimen as measured by VN or HI, or IgG enzyme immunoassay (EIA), OR
- Virus-specific immunoglobulin M (IgM) antibodies demonstrated in serum by IgM antibody-capture enzyme immunoassay (EIA)

METHODS (cont.)

Laboratory Diagnostic Testings
- Serological testing of serum or cerebrospinal fluid (CSF) to detect virus-specific IgM and neutralizing antibodies.
  - immunoglobulin M (IgM)-enzyme-linked immunoassay (MAC-ELISA) and
  - microsphere-based immunoassay (MIA) that utilizes xMAP technology
    - polystyrene microspheres are covalently linked to a flavivirus group-reactive monoclonal antibody
    - IgG-depleted serum and an anti-human IgM phycoerythrin conjugate are added concurrently to the reaction mixture
    - the mixture is incubated, and then the median fluorescent intensities (MFIs) are determined.
    - data transformation Excel add-in program available from CDC.

RESULTS

Table 1. Laboratory testing results of 479 patient specimens by MAC-ELISA and MIA

		MAC-ELISA Results (N=479)					Total no. of Specimens
		Negative	Equivocal	High Bkgd	SLEV	WNV	
		427	12	14	1	25	479
MIA Results (N=479)	Negative	422	10	12	1	*1*	446
	Non-Specific	4	2	2	0	2	10
	SLEV	*1*	0	0	0	*1*	2
	WNV	0	0	0	0	21	21

- One case WNV-IgM weak positive by ELISA but negative by MIA.
  - 64-yro male in Baltimore City
  - symptoms of fever, pain at hip, lower back and shoulder
  - recent travel to St. Thomas.
- One case SLEV-IgM positive by MIA (QNS for WN testing) and WNV-IgM positive by ELISA (QNS for SLEV testing).
  - 57-yro male in Queen Anne's County
  - symptoms of headache, fever, neck stiffness, altered mental status, and muscle weakness
  - travel history unknown.
- One case WNV-IgM weak positive by ELISA but negative by MIA.
  - 64-yro male from South Carolina
  - symptom of muscle weakness
  - no recent travel history.

Table 2. Work-flow Analysis of MIA in Comparison to MAC-ELISA

MIA	MAC-ELISA
Day 1 Prepare reagents for assay: 1 hr Day 2 Remove IgG from serum samples: 1hr Make Dilutions of depleted sera, CSF, and controls: 15min Combine samples and reagents on plate: 30 min Incubate: 1.5hr Read and calculate on Luminex/Bioplex 100: 45min	Day 1 Coat assay plate: 30min Store at 4°C: overnight Day 2 Wash plate: 10min Incubate with blocking buffer: 30min Wash plate: 20min Incubate with specimen or control: 1hr Incubate plate with viral or normal antigen: overnight Day 3 Wash plate: 20min Incubate with monoclonal antibody: 1 hr Prepare plate reader: 15min Wash plate: 25min Incubate with TMB: 15min Add stop solution: 5min Read plate: 10min
Turn-around time: 1-2 days Hands-on time: 5hr	Turn-around time: 3 days Hands-on time: 4hr

SUMMARY

- The WNV/SLEV MIA testing was 95% accurate compared to results obtained from MAC-ELISA testing. (Note: accuracy based on a strict comparison with MAC-ELISA results is not optimal because the MAC-ELISA testing alone is not 100% accurate, especially considering equivocal results.
- MIA reduces turn-around time.
- We intend that the duplex MIA will eventually replace the MAC-ELISA in our laboratory.

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