



June 17, 2004

To all regional laboratories

Re: West Nile virus testing for 2004

Dear Laboratory colleague

We would like to provide you a brief update on our West Nile testing for this year. Attached are a lab testing infosheet, which will be distributed to physicians via Alberta Health and the Regional MOH's, and a table for interpretation of tests that we have provided to AHW. Also attached is the updated history form which we encourage physicians to complete and submit with the requisition and specimens. I would like to draw your attention to a few major points which are departures from last year.

1. Nucleic acid amplification testing (NASBA or PCR) for WNV on plasma was surprisingly effective in 2003. During the first week of illness nearly half the cases are detected by this method, usually prior to the development of antibody. We will therefore offer this test, "WNV PCR", in 2004, and recommend that in addition to the 5-10 ml serum separator tube for WNV serology, that 5-10 ml EDTA blood be submitted acutely for ALL PATIENTS for WNV-PCR.
2. CSF gave a low yield for WNV in 2003, but we will continue to offer WNV PCR on CSF, if requested. Please send 1 ml in a dedicated tube if possible. Refrigeration is recommended, and if delays of >24hours are anticipated, we recommend sending on dry ice. Because we detected enteroviruses in approximately 50 specimens last year, enterovirus PCR will be added automatically if WNV PCR is requested.
3. During the first week of illness, only about 50% of patients develop IgM antibody to WNV. The IgM negative patients are usually viremic however, and will be detected by the PCR on EDTA blood, as above. Together, IgM and PCR detect about 95% of cases on the first blood draw. Convalescent serology will therefore not be routinely recommended for all patients, but could be performed for severe or complex cases.
4. Our preliminary data confirm a previous report that WNV IgM persists into the following year. As most infections are asymptomatic, this makes interpretation of a positive IgM test difficult. To help sort this out, we will be recommending a convalescent serum in two weeks for patients who are IgM positive. Sera will be screened for WNV IgG, and tested for changes in antibody level. Acute cases



have rises in antibody levels, or high hemagglutination titres, while past infections have stable low titres.

As the testing for West Nile virus becomes more complex, we will be adding more interpretive comments to the reports to assist the physician. Attached is a table listing some of the most common comments, which I hope will assist you in preparing your lab information system for the coming season. There will still be occasional complex cases which require a specific free text discussion, and I appreciate your patience with this.

If you have any questions about our West Nile testing, please do not hesitate to call.
Sincerely,

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List of West Nile virus Reporting Comments

June 17th, 2004

Test result:	Comment:
IgM negative on first serum	In up to 50% of patients, WNV antibody does not develop during the first week of illness. These patients are usually viremic, and positive on PCR assay of whole blood (EDTA). Please interpret this negative antibody result in light of duration of illness and corresponding EDTA blood PCR result.
IgM-positive on first specimen	In many patients, WNV IgM may persist at low levels for >1 year. Please submit a follow up serum specimen in two weeks to document rising titres (acute infection) or static titres (past infection).
Significant rise in IgG antibody level	A significant change in flavivirus antibody level was detected, indicating recent flavivirus infection or vaccination. IgG antibody cannot differentiate amongst flaviviruses (e.g. West Nile, St Louis Encephalitis, Japanese Encephalitis, Dengue, Yellow Fever).
Stable, low level antibody	Stable, low levels of flavivirus antibody suggest past exposure to a flavivirus. IgG antibody cannot differentiate amongst flaviviruses (e.g. West Nile, St Louis Encephalitis, Japanese Encephalitis, Dengue, Yellow Fever).
Result on positive plasma PCR	<p>WEST NILE VIRUS RNA *West Nile Virus POSITIVE</p> <p>Detection was undertaken using NASBA primers and probes described previously (Lanciotti et al., 2001). This assay has not been approved by FDA or Health Canada and the result should be interpreted in the clinical context.</p> <p>Result for a second, confirmatory, molecular amplification assay to follow.</p> <p>WEST NILE VIRUS RNA *West Nile Virus POSITIVE</p> <p>Detection was undertaken using the RealArt West Nile LC</p>



	<p>PCR kit (artus-Biotech USA). This assay has not been approved by FDA or Health Canada and the result should be interpreted in the clinical context.</p> <p>This test confirms the previously reported positive result for this sample.</p>
<p>Result on negative plasma PCR</p>	<p>Detection was undertaken using NASBA primers and probes described previously (Lanciotti et al., 2001). This assay has not been approved by FDA or Health Canada and the result should be interpreted in the clinical context.</p> <p>The specificity of the molecular West Nile assays in plasma was 100% during the validation phase. Sensitivity is approximately 50% when plasma is collected during the first 8 days of illness, but is rarely positive thereafter. Results should be interpreted together with WNV serological tests.</p>
<p>Result on positive CSF PCR</p>	<p>WEST NILE VIRUS RNA *West Nile Virus POSITIVE</p> <p>Detection was undertaken using the RealArt West Nile LC PCR kit (artus-Biotech USA). This assay has not been approved by FDA or Health Canada and the result should be interpreted in the clinical context.</p> <p>Result for a second, confirmatory, molecular amplification assay to follow.</p> <p>WEST NILE VIRUS RNA *West Nile Virus POSITIVE</p> <p>Detection was undertaken using NASBA primers and probes described previously (Lanciotti et al., 2001). This assay has not been approved by FDA or Health Canada and the result should be interpreted in the clinical context.</p> <p>This test confirms the previously reported positive result for this sample.</p>
<p>Result on negative CSF PCR</p>	<p>WEST NILE VIRUS RNA West Nile Virus</p>



	<p>NEGATIVE</p> <p>Detection was undertaken using the RealArt West Nile LC PCR kit (artus-Biotech USA). This assay has not been approved by FDA or Health Canada and the result should be interpreted in the clinical context.</p> <p>The specificity of the molecular West Nile assays in CSF was 100% during the validation phase. Sensitivity for West Nile in CSF may be very low, and results should be interpreted in light of the WNV serological assays and plasma RNA testing.</p>
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