West Nile Virus Testing in 2006

West Nile virus testing for mild uncomplicated febrile illness is not required for public health purposes and is generally not indicated unless, in the physician's opinion, the results will influence clinical management. Testing is recommended for the following patients during West Nile season (June – Oct):

- Meningitis, encephalitis, acute flaccid paralysis or other neurological symptoms,
- Patients with unexplained fever occurring more than 3 days and less than 8 weeks after a blood transfusion,
- Febrile patients with a history of blood, organ or tissue donation within 8 weeks,
- Transplant or other immunocompromised patients with unexplained fever and possible exposure to mosquitoes,
- Pregnant women with unexplained febrile illnesses during WNv season
- Healthy blood donors with positive WNv screening tests at Canadian Blood Services.

Please submit to ProvLab the following specimens, with the requisition and **Arboviral History Form** (available at www.provlab.ab.ca):

Specimen:	**Transport:**	**Please specify on requisition:**	**Comment:**
Acute serum (All patients)	7-10 mL in gold top serum separator tube(s)	"WNv - acute"	WNv IgM will be performed within 3 days.
Acute whole blood (All patients)	7-10 mL in purple top EDTA tube(s)	"WNv PCR"	Detects about 40% of cases during 1st week of illness, prior to antibody.
CSF	1 mL in dedicated sterile tube, if possible	"WNv PCR" or "HSV PCR"	Testing for Enterovirus will be done automatically if WNv PCR ordered.
Convalescent serum (>10 days after acute, critical cases only)	7-10 mL in gold top serum separator tube(s)	"WNv-convalescent"	WNv IgM will be repeated, and IgG will be tested to detect seroconversion.

- IgM on serum, and PCR on EDTA blood together detect >95% of cases on the first blood sample. Convalescent serology may be useful for rare critical cases where IgM and PCR are both initially negative.
- Many patients remain IgM-positive for > 1 yr, so a convalescent serum is recommended to demonstrate changing IgG titres in IgM-positive patients.
- WNv PCR can detect viral RNA in CSF, but has low sensitivity (10-20%). Many CSF specimens are positive for enterovirus.

Please call if you have questions or comments
Peter Tilley MD FRCPC (403) 944-1203, p.tilley@provlab.ab.ca

How to Interpret Acute West Nile Virus Test Results

Acute WNv tests:

IgM	IgG	EDTA blood WNv NASBA/PCR	CSF WNv PCR	Interpretation
Any	Any	POSITIVE		This patient is viremic, and is a confirmed case of West Nile virus infection. There is no cross-reactivity with other flaviviruses in the Provincial Lab WNv NASBA/PCR
			POSITIVE	Viral RNA present in the CSF. This is a confirmed case of West Nile virus infection. There is no cross-reactivity with other flaviviruses in the Provincial Lab WNv PCR
			negative	Viral RNA not detected in the CSF. This test has very low sensitivity and does not rule out WNv infection. Please refer to blood tests.
POSITIVE	Negative	negative or not submitted		Possible acute West Nile virus infection, but IgM persists at low levels for 1 year in 60% of patients, and this may be a previous season's infection. Possible non-specific IgM reaction. A follow-up serum in two weeks is recommended to demonstrate rising IgG titres and low avidity IgG. There is very little cross-reactivity with other flaviviruses in IgM tests.
POSITIVE	POSITIVE, high avidity	negative or not submitted		Past West Nile virus infection. IgG antibody takes 3-6 months to mature to the high avidity level. IgM persists into the following season in 60% of patients, and this likely a previous season's infection. A follow-up serum in two weeks is recommended to demonstrate stable titres.
POSITIVE	POSITIVE, low avidity	negative or not submitted		Probable acute West Nile virus infection. IgG antibody takes 3-6 months to mature to the high avidity level. A follow-up serum in two weeks is recommended to demonstrate changing titres and confirm infection
Negative	POSITIVE	negative or not submitted		Past flavivirus exposure. IgG assays cannot differentiate WNv from dengue, St Louis encephalitis, Japanese encephalitis or yellow fever. Could be due to vaccination. IgG does not reliably indicate immunity to WNv.
Negative		negative		Not a WNv case. Data from 2003 show that an IgM test and blood NASBA, performed together on the initial blood sample, detect >95% of cases. Follow-up serology is recommended only for critical cases.

PCR: polymerase chain reaction, NASBA: nucleic acid sequence based amplification. (Both are DNA or RNA amplification tests with similar clinical roles)

How to Interpret Acute and Convalescent West Nile Virus Serology Results

Acute and Convalescent WNv tests:

Acute	Convalescent	Interpretation
IgM negative	IgM POSITIVE	Probable WNv case. IgM is relatively specific for WNv, and a seroconversion indicates that infection is recent (<3 weeks).
IgM POSITIVE IgG negative	IgM POSITIVE IgG POSITIVE, significant rise in IgG level	Probable WNv case. Rising IgG levels, or rising WNv HI titres, or low avidity IgG indicate recent flavivirus exposure. WNv IgM is relatively specific for WNv, indicating recent infection is WNv. First 5 cases in Alberta will be submitted to the National Microbiology Lab for confirmation by PRNT.
IgM POSITIVE IgG POSITIVE	IgM POSITIVE IgG POSITIVE, significant rise in IgG level	
IgM POSITIVE IgG negative	IgM POSITIVE IgG POSITIVE, Fourfold rise in WNv HI titre	
IgM POSITIVE IgG POSITIVE	IgM POSITIVE IgG POSITIVE, Low avidity IgG	
IgM POSITIVE IgG negative	IgM POSITIVE IgG POSITIVE, Fourfold rise in WNv HI titre, WNv PRNT POSITIVE, titre $\geq$80	Confirmed WNv case. WNv PRNT test is highly specific for WNv, indicating definite WNv exposure. Rising HI titre (or rising IgG level, or low avidity IgG) indicate recent infection.
IgM POSITIVE IgG POSITIVE	IgM POSITIVE IgG POSITIVE, Stable IgG level, High avidity IgG	Past WNv infection. IgM persists into the following summer in 60% of patients.
IgM negative IgG negative	IgM negative IgG POSITIVE, significant rise in IgG level	Acute flavivirus infection, probably not WNv. IgG and WNv HI tests also detect St Louis encephalitis, Japanese encephalitis, dengue and yellow fever, including vaccine responses. Needs neutralization titres at National Lab.
IgM negative IgG POSITIVE	IgM negative IgG POSITIVE, Stable IgG level, High avidity IgG	Past flavivirus exposure. IgG and WNv HI tests also detect St Louis encephalitis, Japanese encephalitis, dengue and yellow fever, including vaccine responses. Not a reliable indicator of WNv immunity.
IgM negative IgG negative	IgM negative IgG negative	Not WNv. Lack of antibody to WNv by 21 days after onset of illness is extremely unusual.

PRNT: plaque reduction neutralization titres, HI: Hemagglutination inhibition assay

West Nile Virus Test Summary for Public Health Practitioners

Test Name	Test Format	Test Performance and Interpretation
WNv Nucleic acid testing (NAT)	Also known as polymerase chain reaction (PCR), or NASBA. Detects presence of viral RNA by an amplification method in plasma or CSF.	• Detects RNA in plasma in about 40% of cases during the first week of illness. Rarely positive after 8 days of illness or when IgM appears. • Low sensitivity in CSF, probably <20%. • A positive NAT test is always confirmed by a second NAT test targeting a different gene. • A positive NAT test indicates a CONFIRMED CASE of WNv infection.
WNv IgM	A high volume enzyme immunoassay test (EIA) which detects WNv-specific IgM in serum	• Only positive in about 50% of cases during the first week of illness (NAT testing detects most of the other 50%). WNv IgM is nearly always positive in cases after the first week of illness. • Little cross-reactivity with other flaviviruses. • WNv IgM antibody persists for >9 months in at least two thirds of cases. A patient with a positive WNv IgM result may have had the infection last season!
WNv IgG	EIA for WNv IgG in serum.	• Cross reacts extensively with other flaviviruses, such as St. Louis Encephalitis, Dengue, Japanese Encephalitis and Yellow Fever, including vaccination. • NOT recommended for asymptomatic persons. NOT a reliable marker of immunity to WNv. • Useful to show rising IgG levels in acute and convalescent sera, which

Test Name	Test Format	Test Performance and Interpretation
		are strongly suggestive of recent flavivirus infection or vaccination.
WNv IgG Avidity	Measures strength of antibody binding to WNv.	• Low avidity antibodies indicate recent (<4 months) infection or vaccination with a flavivirus. In combination with a positive WNv IgM result, indicates a PROBABLE WNv CASE. • High avidity antibody indicates a mature response, and exposure to a flavivirus at least 6 months previously.
WNv Hemagglutination Inhibition Titre	Measures ability of patient's antibodies to block binding of WNv to goose red blood cells! Provides a quantitative measure of antibody level (titre). Performed at the National Microbiology Lab in Winnipeg.	• Detects both IgM and IgG. • Cross reacts extensively with other flaviviruses, such as St. Louis Encephalitis, Dengue, Japanese Encephalitis and Yellow Fever, including vaccination. • Useful to show rising antibody levels in acute and convalescent sera, which is strongly suggestive of recent flavivirus infection or vaccination
WNv Plaque Reduction Neutralization Titre (PRNT)	Measures ability of patient serum to block live WNv infection in a cell line. Performed in the Containment Level-3 Lab at the National Microbiology Lab in Winnipeg.	• Highly specific for WNv. "Gold standard" serologic test. • Indicates CONFIRMED previous WNv infection. • Hazardous and laborious. Not a rapid test. Results takes 4-8 weeks.

P. Tilley MD FRCPC
403-944-1203
p.tilley@provlab.ab.ca