Public Health Ontario | Santé publique Ontario

LABSTRACT – May 2021

West Nile Virus Serology Testing

Audience

Healthcare providers, public health units, specimen collection centres, and laboratories involved in submission of specimens for West Nile Virus (WNV) testing.

Overview

1. Guidance on interpretation of WNV serological test results.
2. An overview of confirmatory Plaque Reduction Neutralization Test (PRNT) testing of positives in health regions (effective June 2013)
3. An outline of recommended laboratory investigations and specimen requirements for cases of suspected WNV infection and cases with neurological involvement (effective June 2013).
4. Information on addition of a secondary confirmatory test for WNV IgM reactive results (effective May 2021).

Content

1. **Interpretation of WNV serological test results (refer to Table 1)**

Enzyme-linked Immunosorbent Assays (ELISAs)

The WNV IgM and IgG Enzyme–Linked Immunosorbent Assays (ELISAs) are used as screening tests: a reactive IgM antibody response using ELISA is specific for WNV and is rarely due to cross-reaction with other flaviviruses. As of May 17, 2021, a secondary WNV IgM ELISA test confirms WNV IgM reactive results.

- If a patient has recently travelled to an area endemic for flaviviruses other than WNV, please document this on the laboratory requisition and contact the laboratory to request any follow up investigations if they are WNV IgM positive.
- Any recent tick bites (the vector for Powassan virus, which is endemic in Ontario) should be documented.
- A reactive IgG antibody response using ELISA may be due to infection with WNV or other flaviviruses, (e.g. dengue, St. Louis encephalitis, Japanese encephalitis, Powassan, or yellow fever virus) which may cross react.

Specimen Requirements and test ordering

Acute and convalescent serology (clotted blood or serum) should be submitted to confirm a recent infection.

When ordering serology:

- Use a Public Health Ontario (PHO) Laboratory requisition form and enter "West Nile Virus" under test description
- Indicate that the test is for **suspect West Nile Virus**, symptoms, date of any significant mosquito exposures, symptom onset date, any travel history, and whether the test is for acute or convalescent serology testing.

2. Confirmatory PRNT testing of positives in health regions

Plaque Reduction Neutralization Test (PRNT)

All early season IgM reactive samples will be further tested using the confirmatory plaque reduction neutralization test (PRNT) which is highly specific for WNV. However, PRNT testing is not necessary to make a diagnosis of WNV infection once WNV season is established within the local health region (3 positive PRNT results in an individual health region), at which time a reactive IgM ELISA test is sufficient for laboratory confirmation. In addition, repeat PRNT testing may not be performed if a patient has had a previous reactive PRNT test.

Indeterminate results for any of the WNV assays may be due to the presence of low-level antibodies or non-specific reactions. Therefore, as with all laboratory tests, the results should be interpreted in the context of the clinical history.

Note: During the summer season samples which are IgG positive and IgM negative will not be further tested by PRNT as this indicates a past flavivirus infection, more than several months ago. If further investigation of these samples is required please contact the Customer Service Centre and ask to speak to the microbiologist.

3. Recommended laboratory investigations and specimen requirements for cases of suspected West Nile Virus infection and cases with neurological involvement

For specimen requirements, in addition to clotted blood or serum acute and convalescent serology, submit a CSF sample for serological testing (WNV IgM), which is reactive in the majority of patients with WNV neuroinvasive disease. A positive CSF WNV IgM is sufficient for laboratory confirmation of CNS WNV infection.

Although WNV PCR testing is available, it is not considered a first line test as it is less sensitive than CSF IgM ELISA due to the brief viremia experienced in WNV infection. Please note that PCR testing is not required to confirm the diagnosis, and usually is not necessary. However, under special circumstances, the National Microbiology Laboratory (NML) can perform WNV PCR testing on patients with suspected neuroinvasive disease who are WNV IgM positive. Contact the Customer Service Centre and ask to speak to a microbiologist to request CSF PCR testing.

Table 1: Interpretation of WNV Laboratory Tests**

IgM ELISA	IgG ELISA	PRNT	Interpretation of WNV Tests
Non-reactive	Non-reactive	Not tested	No serological evidence of recent or past WNV infection.
Non-reactive	REACTIVE	Not tested	Probable past WNV and/or other flavivirus infection or vaccination against a non-WNV flavivirus (e.g. yellow fever and Japanese encephalitis virus). PRNT testing is not warranted.
REACTIVE or Indeterminate	REACTIVE	REACTIVE ≥1:40	Consistent with recent or past WNV infection. IgM antibodies may persist for >1 year at low levels and may be indicative of a previous infection.*
REACTIVE	Non-reactive or Indeterminate	REACTIVE ≥1:40	Consistent with recent or acute WNV infection. A follow-up serum sample in two weeks is recommended to demonstrate the development of IgG antibodies. The failure to develop IgG antibodies suggests possible cross-reactive antibodies from another flavivirus infection.
REACTIVE	Non-reactive or Indeterminate	Non-reactive or Indeterminate 1:10 – 1:20	Probable recent or acute WNV infection. A follow-up serum sample in two weeks is recommended to demonstrate the development of IgG antibodies. The failure to develop IgG antibodies suggests a non-specific IgM reaction.
REACTIVE	REACTIVE	Non-reactive	Probable recent or past WNV and/or other flavivirus infection or vaccination. A follow-up serum sample in two weeks is recommended, as well as consideration for testing for other flaviviruses, depending on the clinical history.*
Indeterminate	REACTIVE	Non-reactive or Indeterminate 1:10 – 1:20	Probable past flavivirus infection or vaccination. The IgG ELISA cannot differentiate between members of the Flavivirus genus. A follow-up serum sample in two weeks is recommended, as well as consideration for testing for other flaviviruses, depending on the clinical history.
Indeterminate	Indeterminate	Not tested	West Nile Virus antibody status inconclusive. A follow-up serum sample in two weeks is recommended. Persistent indeterminate results for WNV IgM and IgG antibodies suggest a non-specific reaction.

* Avidity testing may be considered in order to differentiate recent (last few months) from remote infections. This should only be considered in patients with severe illness (e.g. hospitalized).

** Once WNV activity is established in a health region, PRNT testing will no longer be performed for the remainder of the season.

For further information

- Contact the PHO's Laboratory Customer Service Centre at 416-235-6556 or 1-877-604-4567 (toll-free), or by email at customerservicecentre@oahpp.ca
- For PHO's Laboratory specimen collection information and previous Labstracts, refer to publichealthontario.ca/test directory
- The current versions of the PHO Test Requisition and other forms are available at publichealthontario.ca/Requisitions
- To subscribe to receive future Labstracts, register on our website
- To register for Autofax and receive laboratory reports by fax directly from our laboratory information system as soon as they are released, contact the PHO's Laboratory Customer Service Centre.

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