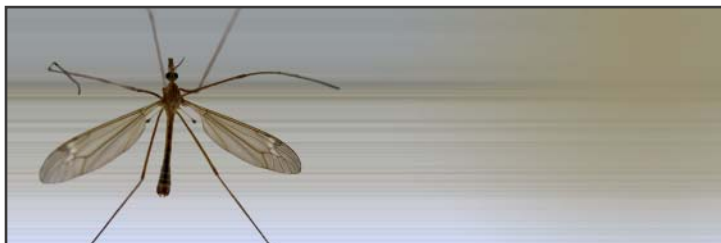


West Nile Virus: 2003 Review

The West Nile Virus (WNV) epidemic of 2002 will be remembered for the significant challenges it presented to clinicians, public health authorities and laboratory scientists. In anticipation of the 2003 season, a review of WNV-related issues is prudent.



OVERVIEW

In 2002, Health Canada reported 325 human cases of West Nile Virus infection, eighteen of which resulted in death. The first case was reported in August and all cases originated in Ontario or Quebec.¹

West Nile Virus is maintained in an enzootic cycle involving culicine mosquitoes and corvid birds (crows, ravens and jays). The virus multiplies in these hosts until the late summer or early fall. When conditions are right, mosquitoes that bite both humans and birds can become infected and pose a threat to humans.² Last year WNV was reported in Saskatchewan birds, and we know that the virus is continuing to move west. As of May 2003 West Nile Virus has not been found in British Columbia.

In Canada and the USA, over 24 cases of transfusion-transmitted cases of WNV infection have been confirmed. As a result, on or about July 1, Canadian Blood Services (CBS) will use nucleic acid technology (NAT) to screen all blood donations for WNV RNA.

CBS has been stockpiling plasma components (shelf-life of 1 year) with the intention of shipping supplies to hospitals just prior to the appearance of the first human case. Red cells (shelf-life 42 days) will be stockpiled in May, to be used during the two weeks after the first human case report, so that unscreened blood will not be used prior to CBS NAT screening.³

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CLINICAL PRESENTATIONS

Only 20% of infected individuals will develop symptoms. These typically include a mild febrile illness lasting 3-6 days associated with headache and myalgia. Some patients develop diarrhea and rash. Only 1 in 150 infected individuals will develop meningoencephalitis. A polio-like syndrome, characterized by asymmetric flaccid paralysis and normal sensory findings has also been described. Advanced age is an important risk factor for neurologic

disease. Prevailing serotype of the virus and background immunity may also dictate manifestations of illness.⁴

DIAGNOSTIC TESTING

For patients with suspected WNV neurological disease the most appropriate specimen is an acute serum sample collected within seven days of illness onset. If available, CSF may be tested for the presence of IgM antibodies and/or NAT. Most laboratories will use an enzyme-linked immunoassay to detect the presence of WNV IgM antibodies. Note that IgM antibodies may be detected a year or more after infection.⁵

Follow-up testing 14 to 21 days after symptom onset is recommended, as many infected patients will not demonstrate antibody responses early during the course of illness. Cross reactivity amongst several viruses of the Flavivirus family, for example St. Louis Encephalitis Virus, will often be reflected in initial serologic testing. For this reason, especially early in the WNV season, positive assays will be confirmed by plaque reduction neutralization assay.⁵

Specific reporting and testing algorithms will be decided at the provincial or health unit level. Samples collected at MDS collection centres will be forwarded to the jurisdiction's WNV testing site (ie public health laboratory), along with the appropriate WNV-specific requisition. Clinicians are reminded to include relevant clinical information as appropriate.

TREATMENT

There is no specific validated treatment for West Nile Virus infection. Studies evaluating hyperimmune globulin and antiviral therapies are anticipated.

WNV Personal Preventative Measures³

Use insect repellent. Spray clothing and exposed skin with mosquito repellents containing $\leq 30\%$ DEET for adults and $< 10\%$ DEET for children. DEET is not recommended for infants younger than 6 months of age or in pregnant women. Wash all treated skin and clothing with soap and water after returning indoors.

Wear long-sleeved clothes and long pants.

Eliminate standing water sources from around homes.

Consider staying indoors from dusk to dawn when mosquitoes are active.

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1. <http://www.hc-sc.gc.ca>
2. Peterson LR, et al. West Nile virus: a primer for the clinician. *Ann Int Med*, 2002;137:173-179.
3. <http://www.bloodservices.ca>
4. Leis AA, et al. A Poliomyelitis-like Syndrome from West Nile Virus Infection *N Engl J Med*, 2002;347(16).
5. Drebot MA, et al. West Nile virus surveillance and diagnostics: A Canadian Perspective. *Can J Infect Dis*, 2003;14(2):105-114.
6. <http://www.peel-bugbite.ca>

Improving the Accuracy of Diagnosis of Vitamin B₁₂ Deficiency with Methylmalonic Acid (MMA) and Homocysteine Assays (Hcy)

The classical case of pernicious anemia described in medical school is easily remembered, just as the metabolic pathways are so easily forgotten. Clinically obvious Vitamin B₁₂ deficiency is now the exception and the current diagnostic problem is the identification of early and sub-clinical deficiencies using imperfect laboratory assays.

Serum Vitamin B₁₂ assays are ordered for the investigation of anemia, tiredness, dementia, peripheral neuropathies etc, but what do the results mean? A low result has a poor predictive value for deficiency and normal values can be seen in pernicious anemia.¹ Even hemoglobins are normal in 28% of cases of pernicious anemia and the MCV is normal in up to 33%. However there is ample evidence that such patients and many others with various neurological disorders have a deficiency state that would benefit from replacement therapy. The dilemma is knowing which assays are true indicators of a deficiency state when as many as 11% of persons over the age of 75 years have reduced levels of Vitamin B₁₂.^{2,3}

The lower the B₁₂ results the more likely there is a true deficiency. Using clinical cut off points improves performance of the assay, but several studies have challenged the value of this approach due to the large numbers of patients with indeterminate results who would in fact respond to vitamin replacement therapy (23%).⁴

Schilling tests and antibodies to intrinsic factor, or parietal cells, have had limited use in the community setting and are being used with diminishing frequency.

Fortunately two metabolic assays, MMA and Hcy, have been demonstrated to have improved diagnostic utility and are now becoming more widely available.

METABOLIC PATHWAYS INVOLVING VITAMIN B₁₂

Vitamin B₁₂ is a cofactor in the conversion of methylmalonic acid to succinyl-coA as well as the conversion of homocysteine to methionine, along with methyl tetrahydrofolate (folic acid). Hence if B₁₂ is deficient there is an accumulation of MMA and Hcy whereas in folate deficiency there is only an accumulation of Hcy (see Figure 1).

Studies have confirmed that virtually all subjects with clinical B₁₂ deficiency will have elevated levels of MMA and Hcy and that these levels will fall with replacement therapy.⁵ MMA and Hcy levels have also been demonstrated to be useful indicators of the clinical importance of low serum B₁₂ levels in those receiving therapy for proven deficiency.⁶

Older patients with B₁₂ deficiency tend to present with neuropsychiatric symptoms in the absence of hematological findings and from 10 – 26 % of cases will have normal B₁₂ levels in the face of elevated MMA and Hcy levels.⁷

DIAGNOSTIC TESTING

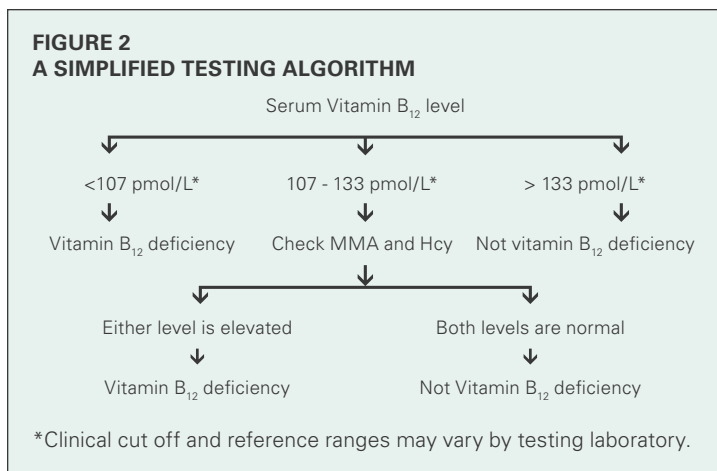
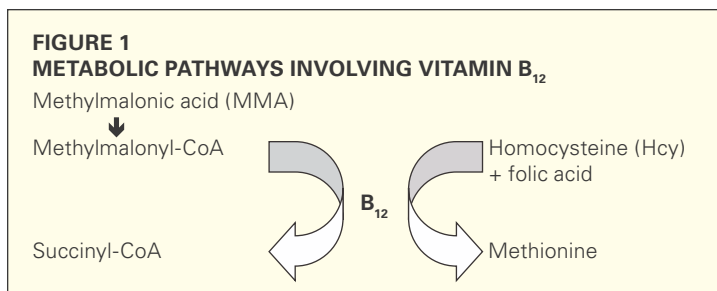
A simplified testing algorithm is proposed in Figure 2 and is intended to improve the diagnostic accuracy of clinical B₁₂ deficiency.⁷ It does not supplant the Vitamin B₁₂ assay as the primary test, although there is literature to suggest that in some cases it would be better to use MMA and Hcy as the primary diagnostic approach.⁵

It should be remembered, however, that as with all assays, other conditions may cause alterations in test results. Elevation of MMA can also be due to renal disease and Hcy elevation is seen

as a consequence of folate deficiency and genetic pre-disposition.

This new assay can be ordered through MDS and results will be available in 2-4 weeks from the time of collection. There are no fasting or special tube handling requirements and the sample is relatively stable. A plasma sample collected in EDTA is required and ideally the plasma should be separated within 6 hours and stored at -20°C for shipment.

Since this assay is not funded by some Provincial Health Insurance Plans, there may be a charge to the patient for testing. For further information please call your local MDS laboratory.



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BC Update

NEW METHOD FOR BIOAVAILABLE TESTOSTERONE

We have implemented an in-house method for performing Bioavailable Testosterone and Free Testosterone. These values correlate more closely to the biologically effective testosterone than the measurement of Total Testosterone or the Free Testosterone "kit" method. However, for routine testing the Total Testosterone and Free Testosterone "kit" methods are recommended as a first step approach. The new test should be reserved for defining borderline cases.

Our approach involves independent measurements of Testosterone, Sex Hormone Binding Globulin (SHBG), and Albumin. These values are submitted to a quadratic equation, developed by Vermeulen et al¹, that uses the binding constants between testosterone and serum proteins to compute the Bioavailable Testosterone and Free Testosterone. It has been validated with a 250 sample comparison with the measured BAT method of Tremblay² (R= 0.94). We prefer the Vermeulen approach because it is more precise and more standardized.

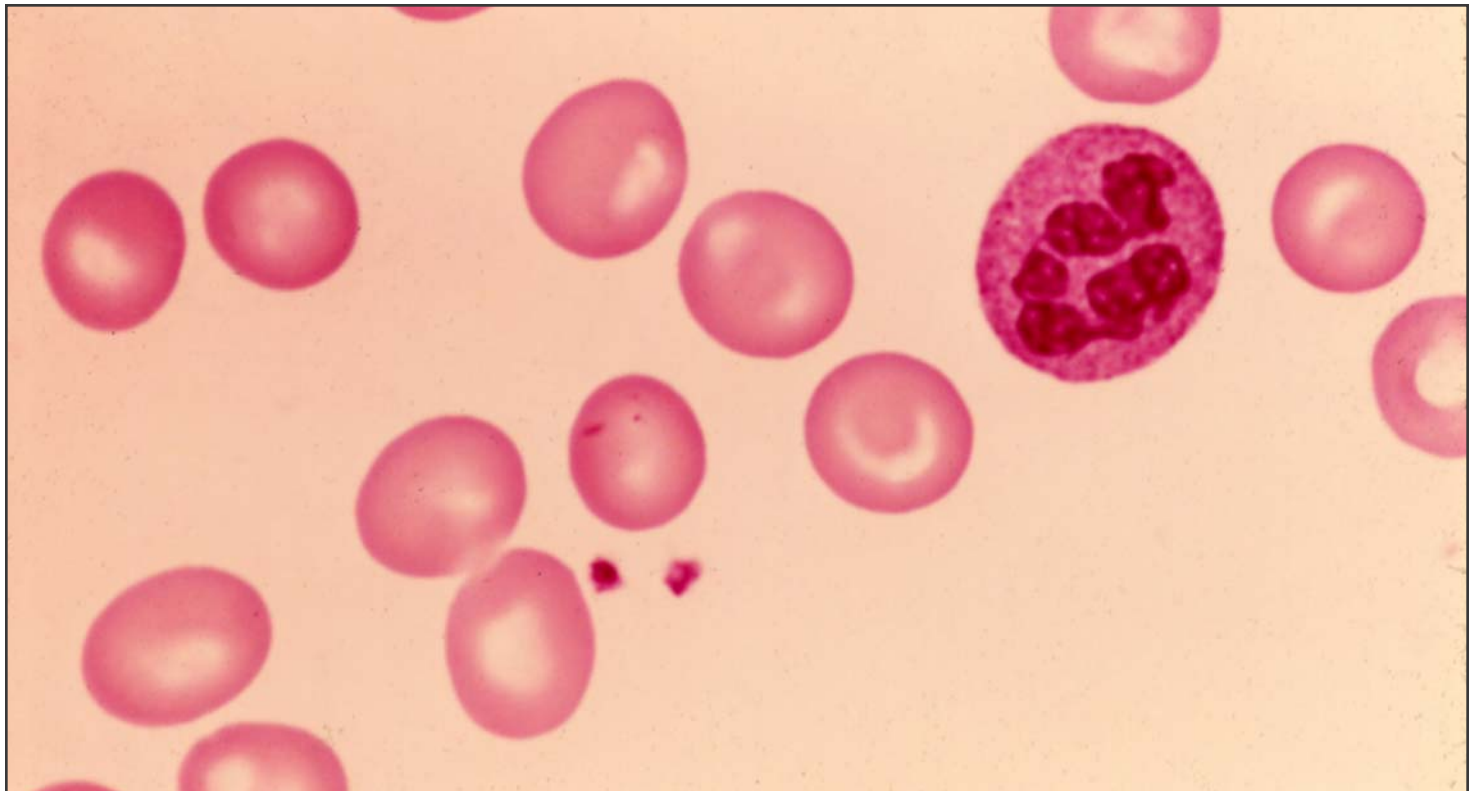
Testosterone testing should be carried out before 10:00 AM, as there is a diurnal variation.

The Bioavailable Testosterone analysis is covered by MSP at the same charge as a Total Testosterone. To obtain the new assay, write "Bioavailable Testosterone" on an MDS Metro requisition. We will return the results listed below. A request for "Free Testosterone" will be interpreted as a request for "kit" method assay as we have done in the past.

References:

1. Vermeulen A, et al. "A critical evaluation of simple methods for the estimation of free testosterone in serum." J Clin Endocrinol & Metab 1999; 84: 3666-3672.
2. Tremblay RR, et al. "Usefulness and limitation of bioavailable testosterone in assessment of androgenicity during the process of aging in men." The Aging Male 1999; 2: 16-21.

| Reference Intervals | Adult Males | Premenopausal Adult Females | Units |
|-------------------------------------|-------------|-----------------------------|--------|
| Total Testosterone | 10.0 - 30.0 | < 4.5 | nmol/L |
| Sex Hormone Binding Globulin (SHBG) | 10 - 70 | 20 - 100 | nmol/L |
| Albumin | 35 - 50 | 35 - 50 | g/L |
| Bioavailable Testosterone (BAT) | 5 - 20 | < 2 | nmol/L |
| Free Testosterone | 200 - 800 | < 70 | pmol/L |



Blood film demonstrating oval macrocytes and a hypersegmented neutrophil in pernicious anemia

TESTING FOR WEST NILE VIRUS IN BC

Surveillance testing for West Nile Virus has begun in BC. Regional health authorities around the province collect dead crows and send them to the Animal Health Centre in Abbotsford for testing. The BC Centre for Disease Control and local governments will be trapping mosquitoes in order to test for the West Nile Virus.

Health care providers should watch for symptoms of WNV in patients and order diagnostic tests on probable cases. The BC Centre for Disease control and regional health authorities will notify the public through the media if WNV is detected in BC.

For more information

Information about West Nile Virus in BC is provided by BC Centre for Disease Control (604-660-5100; web site www.bccdc.org) and the Regional Health Authorities. Health Authority telephone numbers are listed in the blue pages in the telephone book or refer to their web site www.healthservices.gov.bc.ca/bchealthcare/healthauthorities.html.

MDS Metro Medical Microbiologists may be reached through the main lab phone numbers (Mainland: 604-431-5005 or 1-800-431-7206; Island: 250-881-3111 or 1-800-297-7747).

MEDICAL SERVICES PLAN MAKES SIGNIFICANT CHANGES TO BLOOD ALLERGY TESTING

Effective immediately, the Medical Services Plan (MSP) in British Columbia has announced significant changes to its coverage of serum allergy testing (SAIGE = Specific Allergen IgE testing).

These tests will now be paid by MSP only if one or both of the following conditions is met:

- A history of life threatening or severe allergic reaction,
- Presence of generalized skin disease.

This removes the previously allowed reason “debilitating symptoms unresponsive to usual management”.

In addition, MSP will now pay for a maximum of 5 allergens per year per patient (previously 20). More than 5 allergens require a consultation with a laboratory physician (see below).

A few hints may assist in staying under the limit:

- Inhalant allergies can usually be determined from history.
- The most common food allergies are: peanut, fish, egg, milk, wheat, and soy. Many “food allergies” are intolerances due to “delayed type” reactions and blood and skin tests will be negative.

- Ordering mixes may be helpful as they are performed and billed as a single test. A positive mix indicates a reaction with at least one member of a group of allergens. The mixes are: Feather (goose, chicken, duck, turkey), Mite (house dust, *D. pteronyssinus*, cockroach, *D. farinae*), Grass (meadow, rye, *M. fescue*, orchard, timothy), Children’s food (egg white, milk, wheat, peanut, soybean), Nut (peanut, hazelnut, almond, coconut, brazil nut), Seafood mix (cod, tuna, shrimp, mussel, salmon). If the mix is positive it may be necessary to conduct further testing to identify the specific allergenic component(s). Additional testing on the same sample can be arranged (see below).

For patients that do not meet the new restrictions, private payment is an option. The price, payable by the patient at the time of blood collection, is \$20 per allergen with a collection fee of \$15. This cost may be covered by a patient’s private insurance plan.

For further information or to increase the number of allergens required call Dr. Michael McNeely at 250-881-3109 (Victoria) or 1-800-304-4011 (anywhere in BC) and state that you wish to discuss “Allergy Testing”.

CREATININE KINASE REPORTING IN BC

The accepted biochemical markers for assessing myocardial injury are serum troponin (Tn) and Creatine Kinase MB (CK-MB). MDS Metro provides both tests. It is our practice to contact the responsible physician by telephone whenever a positive Tn or CK-MB is found.

CK-Total is measured in order to perform the CK-MB evaluation and

is also used to diagnose and monitor skeletal muscle pathology such as myositis and muscular dystrophy. The CK in these settings is often very abnormal but reporting urgency is not critical.

In order to avoid needless after hours telephone calls, MDS Metro will no longer phone abnormal CK-Total results unless a CK-MB has been ordered and is abnormal.

