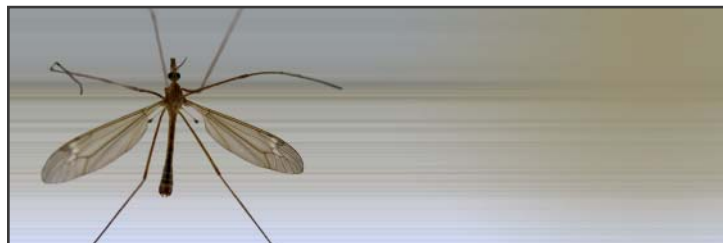


# West Nile Virus: 2003 Review

*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.<sup>1</sup>

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.<sup>2</sup> 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.<sup>3</sup>

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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.<sup>4</sup>

## 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.<sup>5</sup>

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.<sup>5</sup>

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 Measures<sup>3</sup>

Use insect repellent. Spray clothing and exposed skin with mosquito repellents containing ≤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 dawn to dusk 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 B<sub>12</sub> 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 B<sub>12</sub> 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 B<sub>12</sub> 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.<sup>1</sup> 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 B<sub>12</sub>.<sup>2,3</sup>

The lower the B<sub>12</sub> 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%).<sup>4</sup>

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 B<sub>12</sub>

Vitamin B<sub>12</sub> 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 B<sub>12</sub> 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 B<sub>12</sub> deficiency will have elevated levels of MMA and Hcy and that these levels will fall with replacement therapy.<sup>5</sup> MMA and Hcy levels have also been demonstrated to be useful indicators of the clinical importance of low serum B<sub>12</sub> levels in those receiving therapy for proven deficiency.<sup>6</sup>

Older patients with B<sub>12</sub> deficiency tend to present with neuropsychiatric symptoms in the absence of hematological findings and from 10 – 26 % of cases will have normal B<sub>12</sub> levels in the face of elevated MMA and Hcy levels.<sup>7</sup>

## DIAGNOSTIC TESTING

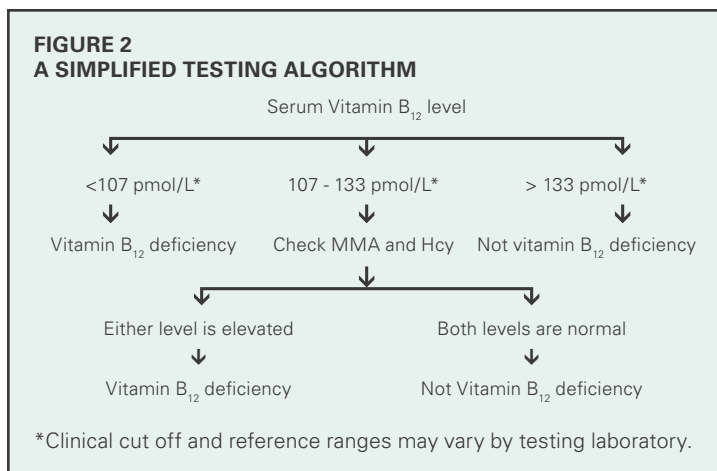
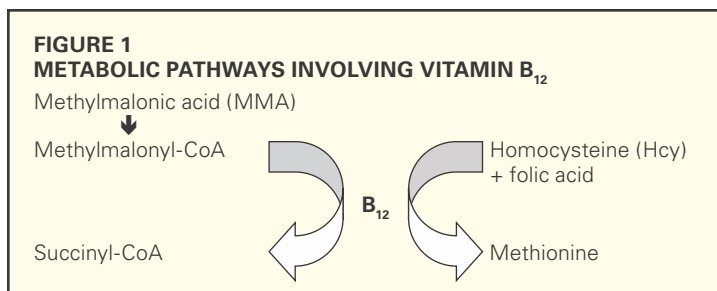
A simplified testing algorithm is proposed in Figure 2 and is intended to improve the diagnostic accuracy of clinical B<sub>12</sub> deficiency.<sup>7</sup> It does not supplant the Vitamin B<sub>12</sub> assay as the primary test, although there is literature to suggest that in some cases it would be better to use MMA and Hcy as the primary diagnostic approach.<sup>5</sup>

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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# Ontario Update

## CHANGES TO THE MDS PROTOCOL FOR REPORTING "ALERT" AND "CRITICAL" VALUES

Experience shows adjustments are required to the reporting protocol introduced in August 2001 to improve clinical utility and to decrease calls, which may not influence patient management. The following changes to the MDS reporting protocol will be implemented. The implementation date will be confirmed in due course. A complete reporting protocol will be distributed prior to implementation.

### Changes to Alert Values

Hemoglobin	80 g/L	(previously 90 G/L)
INR	4.5	(previously 3.6)

INRs between 3.6 and 4.4 will be communicated during office hours and may be transmitted by fax.

### Changes to Critical Values for Glucose and Ketones in Urine

When blood glucose is analyzed on the same day as urinalysis is performed, results for the blood glucose will take precedence and may lead to the downgrading of the critical result in urine. This will only occur in patients whose diabetes is documented in the laboratory by a previous elevated blood glucose or HBA<sub>1c</sub>.

Urine Glucose >55mmol/L with Ketones >1.5mmol/L will always be considered a critical value in children less than 12 years of age or adults with no documentation of a diagnosis of diabetes mellitus in the laboratory.

### Recurrent Alert and Critical Values for Specific Analytes

After successful communication of certain Alert and Critical values, recurrent results in these ranges pertaining to the same patient are not unexpected and further immediate communication is generally not clinically useful.

If within a four-month timeframe, the previous result was an Alert or Critical value, the following test results will not be communicated as part of the protocol, but will be reported the next working day.

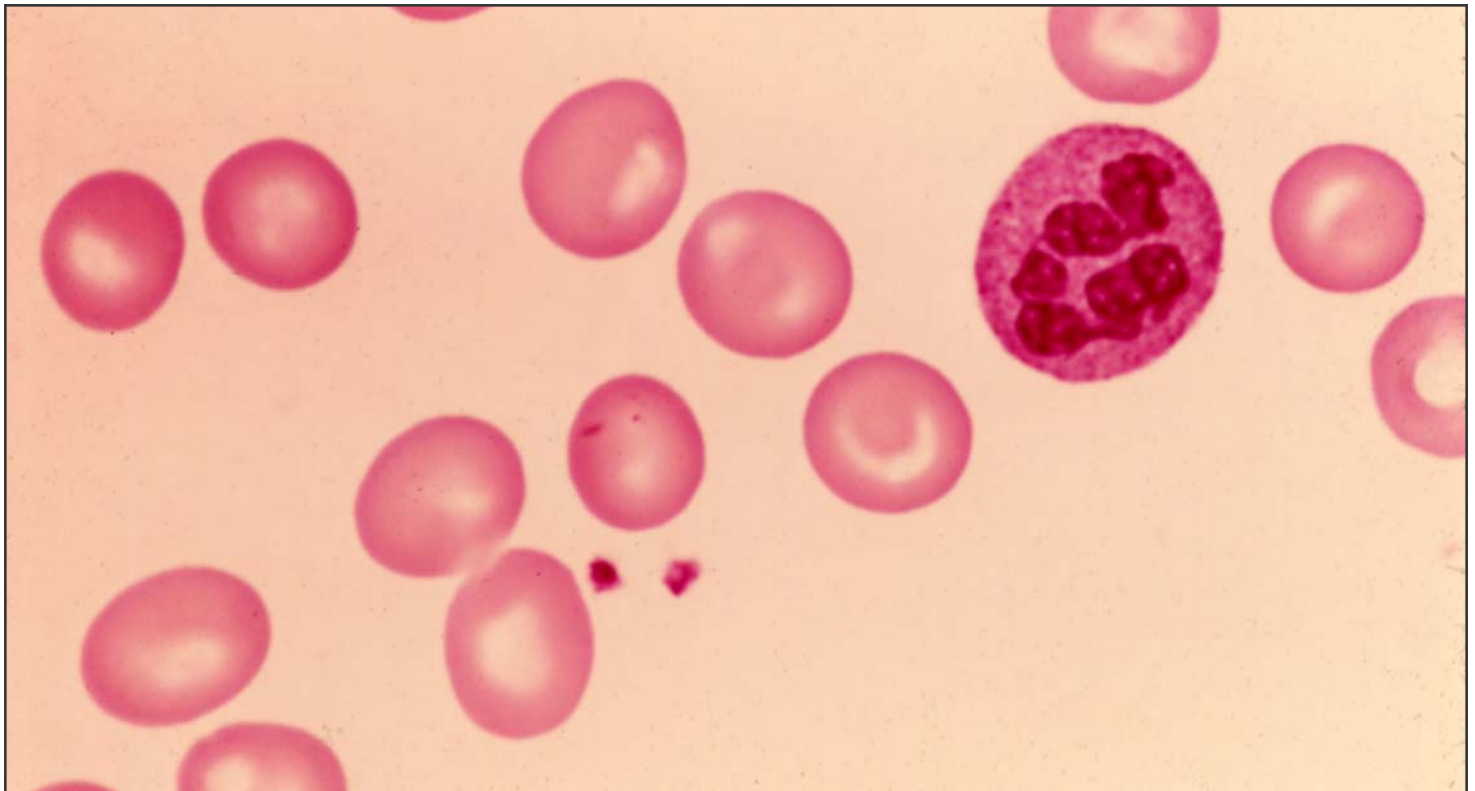
Amylase	Hemoglobin
Calcium	Platelet Count
Ionized Calcium	Total WBC Count
Creatinine	Absolute Neutrophils
Lipase	
Magnesium	
Urea	

Exceptions to this rule are as follows:

1. When hemoglobin has fallen by 10 g/L or more, from the previous Critical value this result will be communicated as Critical.
2. Platelet counts of  $10 \times 10^9/L$  or less will be communicated as Critical even when recurrent.

A clinician requiring immediate communication of known Alert or Critical values listed above may over-ride the rules by requesting the analysis be handled as urgent.

Each year the Critical Values Protocol helps identify patients who require immediate clinical intervention when the seriousness of their condition was not appreciated during clinical examination. You are reminded of your responsibility to provide a 24-hour contact number and provide the information concerning coverage of your practice during your absence.



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

## CLINICAL INFORMATION OPTIMIZES PROCESSING OF PATIENT SAMPLES

Clinicians are asked to include relevant clinical information on specimen requisition forms, allowing the laboratory to appropriately process and report results on submitted specimens.

The table below summarizes essential clinical information related to commonly ordered tests.

TEST	ESSENTIAL CLINICAL INFORMATION
<b>Microbiology</b> All specimens  Wound Swabs/Sterile Sites/Chlamydia Parasitology Blood cultures  Genital	Pregnancy status, immune status, antibiotic allergies, previous failed antibiotic treatments, present antibiotic treatment, if applicable  Body site  Travel history, immune status  Prolonged incubation time required, if applicable (ie endocarditis, Brucellosis, fever unknown origin)  Vaginal, cervical or rectovaginal site
<b>Hematology</b> Heparin  Lymphocyte markers (Flow Cytometry)	Type and name of heparin administered  Complete MDS requisition for Lymphocyte marker analysis. Include clinical diagnosis
<b>Molecular Diagnostics</b> Human Papillomavirus (HPV)	PAP smear history of ASCUS
<b>Therapeutic Drug Monitoring</b> Aminoglycosides / Vancomycin  Other TDM	Peak/Trough collection, if applicable Time since last dose  Time since last dose

## DISCONTINUED TESTING FOR PROSTATIC ACID PHOSPHATASE (PAP)

Prostatic Acid Phosphatase (PAP) has traditionally been used as the primary biochemical marker for prostate cancer. However, it is now widely accepted that prostatic specific antigen (PSA) is a superior indicator and the test of choice. After a critical review of the literature and consultation with stakeholders, MDS Laboratory Services, Ontario, has decided to discontinue testing for PAP.

Please be advised that, effective June 1, 2003, we will no longer be offering this service.

Should you have any questions and/or require further information, please feel free to contact one of the MDS Medical or Scientific Staff at 416-675-0637.

## REFERENCE INTERVAL CORRECTION: RBC FOLATE

In the April 2003 issue of *inside Diagnostics*, the reference interval for RBC folate was incorrectly published as > 164 nmol/L. The

correct reference interval is > 372 nmol/L (or > 164 ng/mL). We apologize for any inconvenience.

