



## VITAMIN B12

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# insight

■ Serum vitamin B12 testing remains the most common vitamin investigation in clinical practice and is included in the investigation of common problems such as anaemia and dementia.

■ Test for Vitamin B12 (item no. 66599) is Medicare rebatable.

### What is vitamin B12?

Vitamin B12 refers to a family of microbially synthesized compounds utilised as cofactors for enzymes that catalyse methyl group transfer. This includes DNA methylation, myelin formation and the conversion of homocysteine to methionine.

### What is the impact of B12 deficiency?

Neurological symptoms in B12 deficiency are paraesthesia of the hands and feet, diminished perception of vibration and position, absence of reflexes, and unsteady gait and balance (ataxia), but the range of symptoms is broad and may include irritability, tiredness, mild memory and cognitive impairment. Severe deficiency causes subacute combined degeneration of the spinal cord. In pregnancy B12 deficiency is associated with a four fold increase in neural tube defects and in childhood is associated with developmental delay and failure to thrive. Unexplained anaemia and/or macrocytosis have traditionally been the indications used for suspicion of B12 deficiency. The presence of hypersegmented neutrophils has been considered to be of high diagnostic accuracy but there are other non-specific causes (eg iron deficiency anaemia).

### Who is at risk of vitamin B12 deficiency?

Only strict vegetarians are considered at serious risk of dietary B12 deficiency, and even then, only after some years. However, vegetarian and vegan diets are becoming increasingly popular. Vegetarians consuming milk products and eggs may have low B12 levels as the B12 content of milk is low (1 mg/L) and even lower if ultra-heat treated. Similarly, breast-fed infants of vegan mothers, if not supplemented, may also be at risk of B12 deficiency. Non animal sources of B12 are extremely limited, with Nori seaweed containing small amounts and B12 levels in mushrooms reflecting compost contamination.

Infants of vegetarian/vegan mothers are also in danger of developing B12 deficiency, even if their mothers do not show any deficiency symptoms. This is due to the relatively high requirement in the rapidly growing child.

### What causes vitamin B12 deficiencies?

Dietary causes of vitamin B12 deficiency are rare in omnivorous populations, given the small amounts of B12 required (1-5 mg/day) and the generally abundant quantities in animal products.

Absorption of B12 requires adequate gastric acid and intrinsic factor as well as a functional terminal ileum. Pernicious anaemia is caused by the autoimmune destruction of the gastric parietal cells and loss of intrinsic factor. Total or subtotal gastrectomy and gastric bypass procedures similarly result in B12 deficiency. Whether age-related atrophy impairs absorption of B12 from food in 30 percent of elderly is controversial. Exocrine pancreatic failure and loss or disease of the terminal ileum may also impede absorption. Intestinal bacterial overgrowth may consume B12 causing deficiency.

### Treatment for Vitamin B12 deficiency

Early treatment may be essential to prevent neurological damage becoming irreversible. Symptoms present for over a year tend to persist. Dietary deficiency can be treated with oral B12 while intramuscular administration is preferable in B12 malabsorption (1 mg daily for one week, then 1 mg weekly for four weeks, thereafter 1 mg monthly for at least six months). Treatment with intramuscular B12 may be required initially for dietary deficiency if the intestinal mucosa has been compromised by a long period of B12 deficiency causing reversible malabsorption.

## Vitamin B12 cont...

### BIOCHEMICAL TESTS FOR DIAGNOSIS of B12 DEFICIENCY

#### Full Blood Examination

While macrocytic anaemia is expected in severe B12 deficiency, more than 25 percent of patients with neurological manifestations of B12 deficiency will have either a normal haemoglobin (Hb), normal mean cell volume (MCV) or both. Absence of macrocytosis in B12 deficiency may also be encountered in patients with concurrent iron deficiency. The most common cause of macrocytosis is liver disease, including alcoholism. Folate deficiency is now a rare cause of macrocytosis.

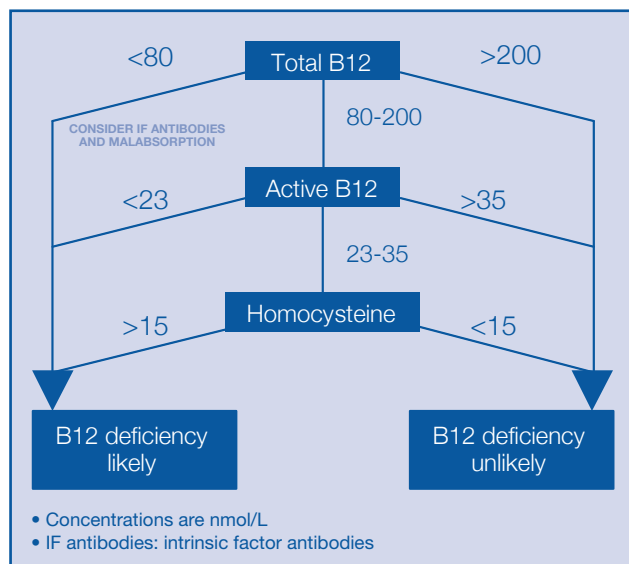
#### Total Vitamin B12 in Serum

B12 is transported in blood predominantly bound to the protein haptocorrin (70%), but it is only the smaller fraction (30%) of B12 transported by transcobalamin that cells have a receptor for. Holotranscobalamin is now considered the best indicator of B12 status ('Active B12').

This is the common test of B12 status however total B12 levels in serum may not reflect tissue status. Non-functional haptocorrin bound B12 is decreased in pregnancy but may be increased in renal failure and haematological malignancies. Therefore tissue deficiency of B12 may occur despite apparently normal serum total B12 levels and vice versa.

#### Active B12 in Serum

The active form of serum B12 (holotranscobalamin) can now be measured directly and Melbourne Pathology has routinely been providing this rebateable service, since early 2007. It is performed in all patients that do not have confidently normal total B12 levels (<200 nmol/L). Up to 30 percent of patients with a normal total B12 (140 - 200nmol/L) have low levels of active B12 indicating B12 deficiency.



Almost all patients with total B12 levels below 80 nmol/L also have low active B12. Our experience confirms that active B12 levels below 23 nmol/L are associated with macrocytosis. Active B12 levels between 23 and 35 may be associated with neurological symptoms and elevated fasting plasma homocysteine.

#### Intrinsic Factor Antibodies

Intrinsic factor antibodies and parietal cell antibodies may be helpful in supporting a diagnosis of pernicious anaemia, but there are limitations with sensitivity and specificity of these tests. B12 treatment for example, can cause false negatives.

#### Homocysteine

The poor conversion of homocysteine to methionine in B12 deficiency can be demonstrated with an elevated fasting plasma homocysteine level. While a normal plasma homocysteine makes B12 deficiency unlikely, elevated homocysteine levels may be seen in the elderly or others with renal disease. Levels may also be elevated in folate and pyridoxine (B6) deficiency.

#### Therapeutic Trial of B12

Where the above tests are equivocal and diagnosis is still suspected clinically, a therapeutic trial of B12 may be considered. In isolated B12 deficiency, substitutional therapy with B12 normalises plasma homocysteine within one to three weeks. If plasma homocysteine remains elevated, folate deficiency must be excluded.



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A graduate of the University of Melbourne, Dr Sikaris trained at the Royal Melbourne, Queen Victoria and Prince Henry's Heidelberg Repatriation Hospitals. He obtained fellowships from the Royal College of Pathologists of

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