Fact Sheet: Vitamin B12 Testing

Background

Vitamin B12 deficiency may be caused by decreased absorption or inadequate dietary intake. Vitamin B12 absorption is a complex process requiring adequate gastric secretion of acid, pepsin, and intrinsic factor, normal exocrine pancreas function, and an intact terminal ileum.

Once absorbed vitamin B12 in circulation is bound to either haptocorrin (~80%) or transcobalamin (~20%). Only the transcobalamin-bound B12 vitamin (termed holotranscobalamin, a.k.a. Active B12) is biologically active. There are certain conditions that increase (e.g. haemat-oncological disorders, solid tumours or liver disease) or decrease (e.g. pregnancy) haptocorrin levels which may result in increased or decreased total B12, respectively.

Vitamin B12 has an important role in erythropoiesis, nerve myelination and the functioning of several enzymes involved in metabolic processes. B12 is a cofactor for methylmalonyl CoA mutase and methionine synthase required for metabolism of methylmalonic acid (MMA) and homocysteine, respectively. Increased MMA and/or homocysteine are sensitive markers of B12 deficiency reflecting the role of vitamin B12 in these pathways.

Investigations for B12 deficiency

Vitamin B12 testing may be indicated in patients with:

- Symptoms suggestive of B12 deficiency:
  - Unexplained neurologic symptoms (peripheral neuropathy, memory/cognitive impairment)
  - Haematological abnormalities – Pernicious anaemia, megaloblastic anaemia, pancytopenia
- Causes of B12 deficiency:
  - Alcoholism
  - Long term dietary deficiency (such as vegans and vegetarians)
  - Malabsorption (gastric or intestinal surgery, inflammatory bowel disease, etc.)
  - Medications that may cause reduced absorption (e.g. metformin, proton-pump inhibitors, H2 blockers).
  - Nitrous oxide abuse

There is currently no gold standard test for establishing B12 deficiency. The current MBS schedule requires testing of total B12 initially with further assessment of holotranscobalamin or functional markers (MMA or homocysteine) in indeterminate cases.

Total B12

Current Vitamin B12 assays measure total B12, bound to haptocorrin and transcobalamin. Serum total B12 testing is relatively insensitive in detecting vitamin B12 deficiency and improved detection can be achieved with measurement of Holotranscobalamin or functional markers such as MMA or homocysteine.

In addition, intrinsic factor antibodies (IFA), which may be present in cases of pernicious anaemia, may cause interference with the B12 assay resulting in artefactual increase in total B12, potentially masking B12 deficiency.

High total B12 may be seen in conditions where there is an increased production of haptocorrin (see above). In such cases there may be a functional B12 deficiency. In such cases, if there are symptoms/signs of B12 deficiency, assessment of functional markers (MMA and/or homocysteine) is recommended.
Fact Sheet: Vitamin B12 Testing

Holotranscobalamin (a.k.a. Active B12)

Holotranscobalamin represents the biologically active fraction of B12. Based on ROC analysis using raised MMA as a surrogate for B12 deficiency, holotranscobalamin appears to be a better marker of vitamin B12 deficiency than total B12. However, there is no single cut off to indicate B12 deficiency with absolute sensitivity and specificity and there is a grey zone of indeterminate results that need further confirmatory testing (see below algorithm).

Methylmalonic acid (MMA) and Homocysteine

MMA and homocysteine are considered functional tests for B12 deficiency, and increased levels are sensitive markers of tissue B12 deficiency. MMA is relatively specific for B12 deficiency, although it is increased in renal impairment and may be increased in small bowel bacterial overgrowth, burns and intravascular volume contraction.

Homocysteine is a less specific marker, also increased in folate deficiency, B6 deficiency, the genetic condition homocystinuria, in certain forms of MTHFR gene polymorphism, as well as in renal impairment. Additionally, it may be falsely elevated by delayed separation of serum/plasma from cells and therefore requires a special collection tube containing a preservative.

MMA and homocysteine tests by liquid chromatography-tandem mass spectrometry are available through NSW Health Pathology Prince of Wales Hospital laboratory.

Algorithm for investigation of B12 deficiency

* Intrinsic factor antibodies (IFAb), which may be present in cases of pernicious anaemia, may cause interference with the B12 assay resulting in falsely increased vitamin B12. If clinical presentation suggests B12 deficiency, consider testing for IFAb, anti-parietal cell antibodies and/or assessment of functional markers of B12 deficiency (MMA/Homocysteine).
Fact Sheet: Vitamin B12 Testing

Key points for diagnosing B12 deficiency

- There is no gold standard test for diagnosis of B12 deficiency.
- Holotranscobalamin is thought to represent the biologically active form of B12 in circulation and may be a better marker of B12 deficiency than total B12.
- Methylmalonic acid (MMA) is often considered a surrogate gold standard for assessing B12 deficiency, but may be increased in renal impairment.
- Homocysteine is an additional functional marker of B12 deficiency which is less specific than MMA and may be elevated in folate deficiency, B6 deficiency, renal impairment or inborn errors of metabolism.
- Use of a combination of the above tests is likely to provide the best assessment of B12 status. In certain cases a trial of B12 supplementation with monitoring of clinical response may be required.

Investigation of high B12 results

High Total B12 may be due to:

- Increased production of haptocorrin which may occur in acute inflammatory conditions, myeloproliferative disorders or solid tumours
- Reduced clearance of B12 due to liver or renal disease
- Release of B12 from the liver in acute liver injury

If the cause for increased B12 is not obvious, for example due to excess intake, investigation of the above causes should be considered.

Sample requirements

B12, holotranscobalamin and MMA: Gold top serum gel tube (as for general biochemistry tests)

Homocysteine: Homocysteine Detection Tube (HDT) available in the collection department at St George, Prince of Wales and Sutherland Hospital. For obtaining correct results, the tube must be filled to the mark indicated on the side, with transport to the laboratory as soon as possible after collection (specimen is stable for up to 4 hours at room temperature and up to 72 hours at 4 degrees).

Testing frequency:

Total B12: 24 hours per day
Holotranscobalamin: Twice weekly
MMA and homocysteine: Once per week

Send samples to:

NSW Health Pathology, Department of Clinical Chemistry and Endocrinology
Level 4 Campus Centre, The Prince of Wales Hospital
Barker Street, Randwick, NSW 2031
Tel: 02 9382 9082

Enquiries:

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Fact Sheet: Vitamin B12 Testing

References:


