AUSTRALIAN Clinicallabs

Vitamin B12 Testing

By Dr David Deam

Vitamin B12 is an important vitamin and deficiencies can be seen despite the availability of nutritious food.

Vitamin B12 is involved as a cofactor for two important enzymes, methionine synthetase and methylmalonyl CoA-mutase. (Fig 1)

Deficiency of B12 can interrupt these key pathways, with consequent disruption of DNA synthesis resulting in megaloblastic anaemia and other adverse effects on the nervous system and other organs.

Vitamin B12 is not manufactured in humans but is absorbed from the diet. This is a complex process which requires gastric secretion of acid, pepsin, and intrinsic factor, normal pancreatic function, and an intact terminal ileum.

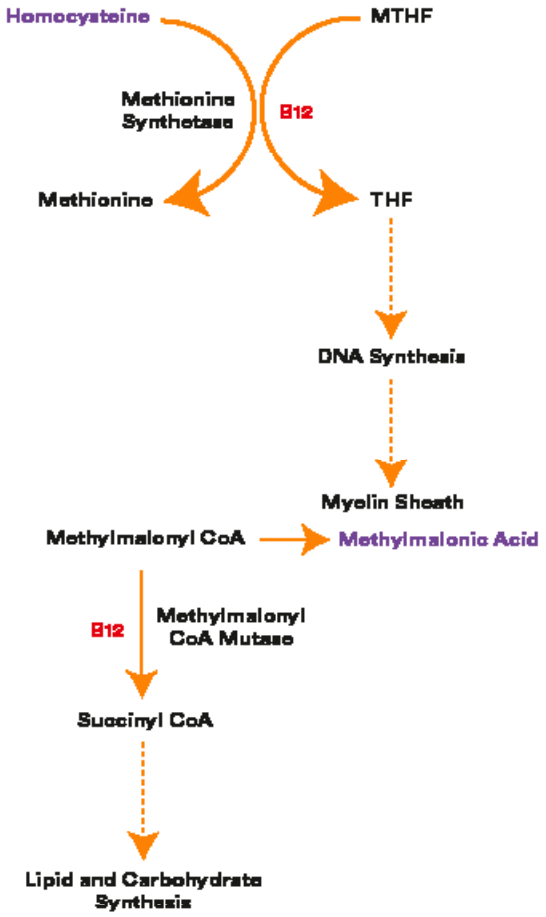

Figure 1. Enzyme reactions involving vitamin B12.

After absorption, vitamin B12 is bound to either haptocorrin (~80%) or transcobalamin (~20%). Only the transcobalamin-bound B12 vitamin (termed holotranscobalamin, or Active B12) can easily enter cells and is biologically active. (Fig 2)

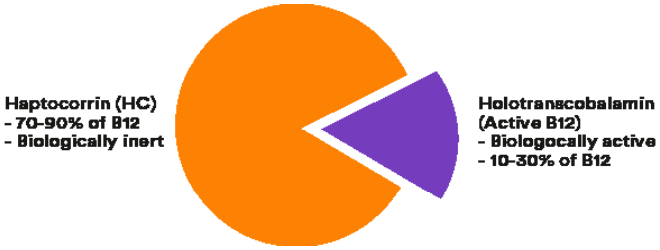

Figure 2. Serum B12 distribution amongst its binding proteins.

The role of the haptocorrin B12 component in the blood is still unknown. Haptocorrin levels may be affected by haemato-oncological disorders, solid tumours or liver disease which can increase levels or other conditions, such as pregnancy that can decrease levels.

Vitamin B12 testing

The most important component of B12 is its levels in tissues where it is utilised in the reactions described above. Although there is no gold standard test to define B12 deficiency, there are a number of tests to assess B12 activity. These include:

1. Tests of B12 levels

a. **Total vitamin B12**

This can be useful in more severe cases but overall it has poor discriminative ability for determining vitamin B12 deficiency. It can give misleading results due to the effect of the inactive haptocorrin-bound B12. This has resulted in our laboratory using an equivocal zone to help interpret B12 levels.

Normal levels	> 180 pmol/L
Borderline	150 – 180 pmol/L
Deficient	< 150 pmol/L

b. Holotranscobalamin (Active B12)

This measurement has a better correlation to tissue B12 levels and is an improved marker of B12 deficiency.

According to the MBS schedule, this test can be done where the initial serum vitamin B12 result is low or equivocal. Our laboratory will run this test automatically if these conditions are met and we have a sufficient, suitable sample.

Normal levels	> 35 pmol/L
Borderline	30 -35 pmol/L
Deficient	< 30 pmol/L

2. Tests of B12 metabolism

Reduced function of enzymes where B12 is a cofactor can cause accumulation of precursor compounds (Fig 1).

a. **Homocysteine**

Increased levels can be seen with B12 deficiency. Unfortunately, there are also other causes of raised homocysteine levels including folate and B6 deficiency as well as with some enzyme defects.

b. **Methyl Malonic Acid (MMA)**

Other factors such as renal impairment and enzyme defects can also influence the results.

Note: There is an out of pocket expense for this test.

3. Antibody tests

Anti-gastric parietal cell and Anti-intrinsic factor antibodies can be detected in autoimmune causes of B12 deficiency.

When to test B12 levels

Testing vitamin B12 levels is mainly done to diagnose or assess B12 deficiency. This can be useful if there are clinical features of possible B12 deficiency; these can include:

- Haematological abnormalities such as megaloblastic anaemia, pernicious anaemia and anaemia of uncertain origin
- Neurological abnormalities such as peripheral neuropathy, polyneuropathy, cognitive decline and dementia
- Possible malnutrition
  - Malabsorption (Coeliac disease, IBD)
  - Poor diet (including vegans and vegetarians)
  - Alcoholism
  - Post bariatric surgery
- Drug effects
  - Metformin
  - Proton-pump inhibitors
  - H2 blockers
  - Nitrous oxide abuse
- Non-specific symptoms
  - Fatigue, weakness
  - Depression
  - Difficulty walking
  - Glossitis
  - Various neurological changes

The incidence of low vitamin B12 levels appears to increase with age (> 65 years).

Interpreting B12 results

Our laboratory will automatically perform an active B12 test if the total B12 level is in a range where B12 deficiency is possible.

If the patient has both total B12 and active B12 levels within the reference range, then B12 deficiency is unlikely.

If the active B12 level is low, then it is likely that B12 deficiency is present, even if the total B12 level is within the reference range.

Note: B12 deficiency can sometimes be present even with normal levels of B12 and active B12. If there is a strong clinical suspicion, a trial of B12 treatment may be warranted.

Treatment

B12 treatment may be given to patients in a variety of formats. The best one will depend on the severity of symptoms, the degree of deficiency and the aetiology of the deficiency.

1. Dietary

If the diet is inadequate in B12 then dietary advice can be given. For vegans where this is not an option, there are some foods which are fortified with B12. These include some soy milks, yeast spread, and vegetarian meat substitutes.

2. Oral B12 supplements

Various supplements are available which contain a range of levels of B12 from 10 to 1,000 mcg. The type and dose to use will depend on the cause of the B12 deficiency and the frequency of use. The higher dose forms can also sometimes be useful in pernicious anaemia patients where enough B12 can be absorbed by non-intrinsic factor mediated means.

3. IM B12 injections

Various forms of parenteral B12 are available. The usual dose is in the order of 1,000 mcg with a dosage interval varying between every few days to every few months depending on the degree of deficiency, the urgency of treatment and whether the patient is undergoing initial treatment or maintenance therapy.

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Dr Deam graduated with Honours in Medicine from Monash University in 1978 and obtained his FRCPA in 1985, following postgraduate training in Biochemistry at the Royal Melbourne Hospital. After several posts in Chemical Pathology at the Royal Melbourne Hospital and the Royal Women's Hospital, he was appointed Head of Chemical Pathology at the Royal Melbourne in 1996. He joined Gribbles Pathology (now Australian Clinical Labs) in 1998. Dr Deam has played an active role in teaching scientific, nursing and medical staff at both undergraduate and postgraduate levels and has been an examiner for the Australasian Association of Clinical Biochemists as well as the Royal College of Pathologists of Australasia. Dr Deam's research interests and publications include work on thyroid function testing, various aspects of diagnostic protein measurement and the rational use of biochemical tests.

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