

Laboratory assessment of vitamin B₁₂ status

Dominic J Harrington^{1,2}

¹The Nutristasis Unit, St Thomas' Hospital NHS Foundation Trust, London, UK
²Division of Women's Health, School of Medicine, King's College London, London, UK

Correspondence to

Dr Dominic J Harrington, The Nutristasis Unit, 4th Floor North Wing (Viapath), St Thomas' Hospital NHS Foundation Trust, Westminster Bridge Road, London SE1 7EH; UK; Dominic.Harrington@viapath.co.uk

Received 8 January 2016
 Revised 19 April 2016
 Accepted 20 April 2016
 Published Online First
 11 May 2016

ABSTRACT

The detection and correction of vitamin B₁₂ (B₁₂) deficiency prevents megaloblastic anaemia and potentially irreversible neuropathy and neuropsychiatric changes. B₁₂ status is commonly estimated using the abundance of the vitamin in serum, with ~148 pmol/L (200 ng/L) typically set as the threshold for diagnosing deficiency. Serum B₁₂ assays measure the sum of haptocorrin-bound and transcobalamin-bound (known as holotranscobalamin) B₁₂. It is only holotranscobalamin that is taken up by cells to meet metabolic demand. Although receiver operator characteristic curves show holotranscobalamin measurement to be a moderately more reliable marker of B₁₂ status than serum B₁₂, both assays have an indeterminate range. Biochemical evidence of metabolic abnormalities consistent with B₁₂ insufficiency is frequently detected despite an apparently sufficient abundance of the vitamin. Laboratory B₁₂ status markers that reflect cellular utilisation rather than abundance are available. Two forms of B₁₂ act as coenzymes for two different reactions. Methionine synthase requires methylcobalamin for the remethylation of methionine from homocysteine. A homocysteine concentration >20 µmol/L may suggest B₁₂ deficiency in folate-replete patients. In the second B₁₂-dependent reaction, methylmalonyl-CoA mutase uses adenosylcobalamin to convert methylmalonyl-CoA to succinyl-CoA. In B₁₂ deficiency excess methylmalonyl-CoA is hydrolysed to methylmalonic acid. A serum concentration >280 nmol/L may suggest suboptimal status in young patients with normal renal function. No single laboratory marker is suitable for the assessment of B₁₂ status in all patients. Sequential assay selection algorithms or the combination of multiple markers into a single diagnostic indicator are both approaches that can be used to mitigate inherent limitations of each marker when used independently.

INTRODUCTION

The clinical utility of approaches taken by laboratories for the assessment vitamin B₁₂ (B₁₂) status are generally poorly understood by service providers and by their users alike. Consequently, opportunities to diagnose B₁₂ deficiency are often not realised. This best practice review highlights the inherent advantages and limitations of diagnostic markers of B₁₂ status and makes recommendations for the application of laboratory assays when used independently and in combination.

BACKGROUND

B₁₂ is essential for the transformation of methyltetrahydrofolate to tetrahydrofolate for DNA synthesis and for fatty acid metabolism. The timely detection and correction of B₁₂ deficiency prevents megaloblastic anaemia and potentially irreversible neuropathy and neuropsychiatric changes.^{1 2}

Crucially, patients who have developed peripheral neuropathy or subacute combined degeneration of the cord may have no discernable haematological diathesis.³

Of at least four distinct laboratory markers of B₁₂ status, it is the total abundance of B₁₂ in serum that has habitually been used for many years by the majority of laboratories. However, automated assays for holotranscobalamin (marketed as 'active B12') have recently become available offering an alternative approach for the evaluation of B₁₂ status. Holotranscobalamin is the form of B₁₂ taken up by cells to meet metabolic demand.

Assays for sensitive functional markers of B₁₂ status, such as the determination of total homocysteine (tHcy) and methylmalonic acid (MMA) in serum, have also been automated. These can be used to detect subtle disturbances in B₁₂-dependent metabolic pathways that stem from B₁₂ insufficiency several years before deficiency-induced pathologies materialise. Unfortunately, it is not yet possible to reliably triage which asymptomatic patients flagged as B₁₂-insufficient using contemporary laboratory diagnostics will ever go on to develop a deficient state of clinical significance. This dilemma is illustrated by a 1.0–3.9-year follow-up study of 432 individuals not treated with B₁₂ after an initial observation of an elevated MMA concentration (defined in the study as >280 nmol/L) showed a longitudinal variation in levels of 34%. As the study progressed, a substantial increase in MMA concentration was detected in only 16% of participants, whereas 44% showed a decrease.⁴ The imperfect prognostic utility of MMA is set against the readiness with which B₁₂ insufficiency may be corrected, and an increasing emphasis in healthcare on disease prevention rather than disease treatment. Low-dose preparations of B₁₂ are available; for example, oral cyanocobalamin (BNF, 50 µg) is licensed within the UK and may 'normalise' laboratory markers of B₁₂ in patients,⁵ with the caveat that it is essential to guard against the suboptimal treatment of latent and emerging pernicious anaemia.⁵

LABORATORY MARKERS OF VITAMIN B₁₂ STATUS

A progressive decline in B₁₂ status initially decreases the abundance of holotranscobalamin in the circulation. Subsequently, tissue stores of B₁₂ are used and begin to diminish, leading to impaired performance of B₁₂-dependent pathways and elevations in serum concentrations of the metabolites tHcy and MMA. Ultimately, an advanced deficient state manifests pathologically. The serum concentration of B₁₂ responds slowly to a deterioration in B₁₂ status.



CrossMark

To cite: Harrington DJ. *J Clin Pathol* 2017;**70**:168–173.

FIRST-LINE ASSESSMENT OF B₁₂ STATUS IN THE LABORATORY

The serum B₁₂ and holotranscobalamin assays have both been highly automated using competitive binding luminescence technologies, making them suitable for the large-scale screening of B₁₂ status in patient populations. tHcy assays are also available using these technologies but poor preanalytical sample stability limits utility. More rarely, automated MMA assays are available using liquid chromatography-mass spectrometry-based platforms.

SERUM B₁₂ ASSAYS

Screening of patients with macrocytic anaemia

The serum B₁₂ assay is appropriate for the screening for B₁₂ deficiency in patients with macrocytic anaemia. A serum B₁₂ concentration below the lower limit of the laboratory reference range is sufficient to diagnose B₁₂ deficiency.^{5 6} In these patients, the abundance of B₁₂ has decreased to a point at which DNA synthesis has become impaired and cell nucleus maturation impeded.^{7–10} The net impact of these pathological changes may be an increase in the mean red cell volume (MCV) of up to 130 fl. If on investigation a serum B₁₂ concentration within or above the reference range is found, then folate deficiency should be considered since it is the interplay between folate and B₁₂ that is responsible for the megaloblastic anaemia seen as a consequence of both vitamin deficiencies. B₁₂-independent causes of an increase in MCV have been widely described.⁹ A coexisting iron deficiency or thalassaemia trait complicates interpretation because macrocytic changes may be masked.⁹

Utility as a screening test in mixed patient populations

The majority of patients with suspected B₁₂ deficiency do not have anaemia—and it is these patients that present the greatest diagnostic challenge. Common neurological symptoms include symmetric paresthesias, numbness and gait problems.^{3 11} It is not known why the severity of megaloblastic anaemia does not correlate with neurological dysfunction.^{3 11} Other symptoms of B₁₂ deficiency include pallor, oedema, pigmentary changes in the skin, jaundice, impaired vibration sense, impaired position and cutaneous sensation, ataxia and weakness. It is essential to note that because serum B₁₂ assays provide an estimate of total B₁₂ abundance rather than direct evidence of metabolic utilisation, it is not possible to confidently exclude B₁₂ deficiency when results fall in the indeterminate range of 125–250 pmol/L. This inherent limitation is clearly evidenced by the evaluation of a serum B₁₂ assay for the detection of B₁₂ deficiency against an MMA cut-off of 750 nmol/L (a concentration indicative of 'definite' B₁₂ deficiency) in which sensitivities of ~35% and ~85% were found at 125 and 250 pmol/L, respectively. The corresponding assay specificity was ~95% and ~45%, respectively.¹² To put this into context, the lower serum B₁₂ cut-off for diagnosing B₁₂ deficiency is typically set at ~148 pmol/L (200 ng/L).¹³ In practice, detectable disturbances in metabolic networks consistent with possible B₁₂ deficiency occur as high as 300 pmol/L.¹⁴ Up to 45% of B₁₂-deficient patients may be overlooked when serum B₁₂ assays are used in isolation.¹⁴ Further investigation using a second-line test is necessary for serum B₁₂ results that fall within the indeterminate range.

Physiological variation in serum B₁₂ concentration and impact on result interpretation

The difficulty of establishing B₁₂ status during pregnancy serves to emphasise commonly faced, but not universally recognised,

physiological changes that impact on serum B₁₂ assay result interpretation. The recommended daily allowance for B₁₂ increases during pregnancy from 2.4 to 6.0 µg/day, but a commonly seen decrease of almost 50% by the third trimester in serum B₁₂ levels is more likely to be a consequence of haemodilution and a fall in the abundance of haptocorrin, one of the two primary B₁₂-binding proteins, than signalling a tissue deficiency as a consequence of increased demand.¹⁵ Although a corresponding small increase in MMA levels may also be seen during pregnancy and post partum, authors question whether this is caused by an increased metabolic rate during pregnancy and lactation rather than indicative of B₁₂ deficiency.¹⁵

Variation in the abundance of B₁₂-binding proteins occurs in the wider population too, with up to 15% of patients with low serum B₁₂ likely to have a low haptocorrin level rather than B₁₂ deficiency.

Interpretation results >1000 pmol/L

Serum B₁₂ levels >1000 pmol/L are not uncommon. With the exception of ongoing B₁₂ replacement regimens, an unexpectedly high concentration of B₁₂ in serum can frequently be traced to changes in the abundance of B₁₂ binding proteins.

An increase in the abundance of haptocorrin is a feature of some malignant diseases including chronic myeloid leukaemia, polycythaemia vera and some solid malignant tumours. This phenomenon has been exploited diagnostically with the abundance of B₁₂-unsaturated binding proteins (unsaturated vitamin B₁₂ binding capacity (UBBC)) used as a marker of fibrolaminar hepatoma.¹⁶ Manual methods to determine UBBC are available.¹⁷ The reference range for UBBC is 670–1200 ng/L;¹⁸ for plasma collected into EDTA-sodium fluoride, it is 505–1208 ng/L;¹⁹ for haptocorrin, 49–132 ng/L; and for transcobalamin, 402–930 ng/L.

An increased level of serum B₁₂ can also be caused by the presence of autoantibodies against transcobalamin, which does not appear to be related to the development of B₁₂ deficiency.²⁰ High B₁₂ levels may also be a consequence of immunoglobulin-complexed B₁₂, resulting in assay interference.

Analytical challenges associated with serum B₁₂ assays based on competitive binding luminescence technologies

Automated assays for the measurement of serum B₁₂ are based on competitive binding luminescence technologies. In the presence of high-titre anti-intrinsic factor antibodies in serum from patients with pernicious anaemia, assays based on competitive binding luminescence technologies generate spurious results.²¹ A study illustrated failure rates of serum B₁₂ assays in the analysis of samples from patients previously diagnosed unequivocally with pernicious anaemia. Also, 6 of 23 (26%) patients were missed by the *Beckman Coulter Access assay*, which used the UniCel DxI 800 Immunoassay System, 5 of 23 (22%) by the *Roche Elecsys Systems Modular Analytics E170* and 8 of 23 (35%) by the *Siemens Advia Centaur assay*.²¹

Assay calibration

The use of assay calibrants that are traceable to metrological standards assists with assay harmonisation and the meaningful comparison of population data. The WHO International Standard for serum B₁₂, 03/178, was ratified in 2007²² as a consensus of B₁₂ protein-binding assays. However, poor alignment of the seven main analytical platforms continues. [Figure 1](#) shows consistent manufacturer-specific assay bias for serum B₁₂ analysis. It is essential that laboratories adopt reference ranges that are compatible with their chosen platform.

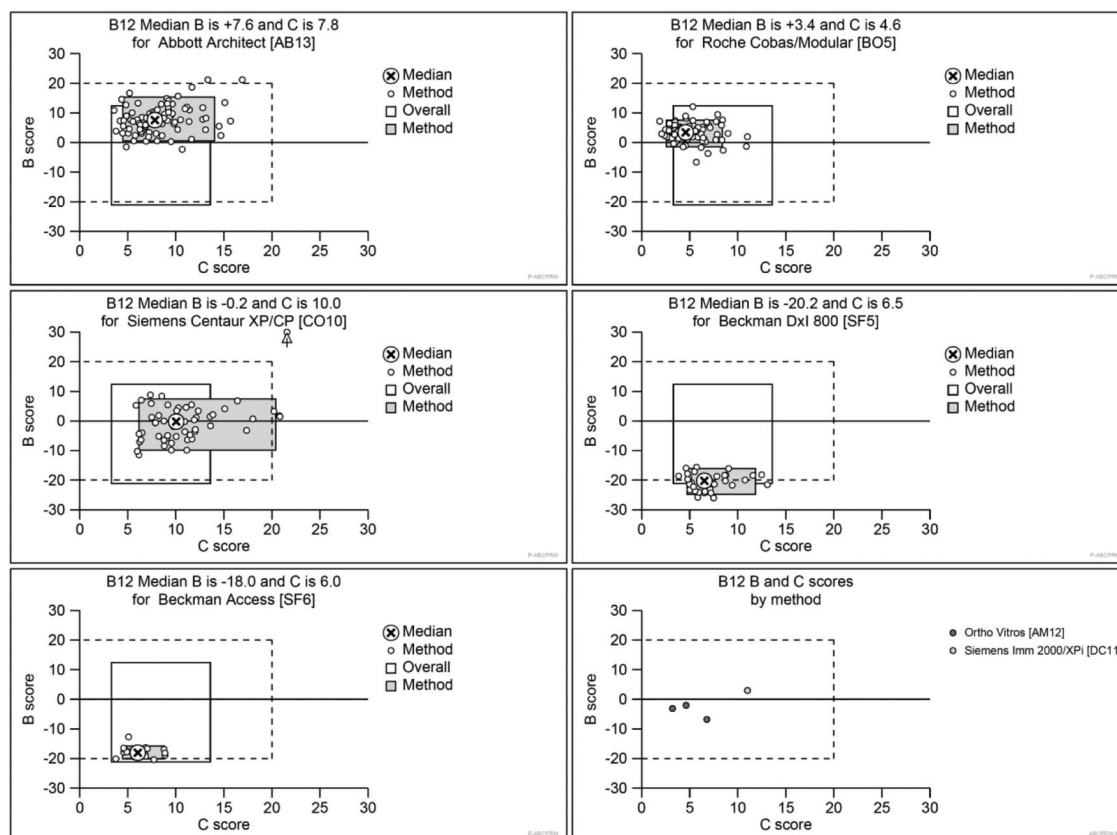


Figure 1 Data from the UK NEQAS Haematinics Scheme showing performance calculated over a rolling window of 6 months (18 External Quality Assurance specimens circulated) by the seven analytical methods used for the analysis of vitamin B₁₂ in serum. Methods clockwise from top left (UK NEQAS method abbreviation): Abbott Architect (AB13); Roche Cobas/Modular (BO5); Beckman Dxl 800 (SF5); Ortho Vitros (AM12) and Siemens Immulite 2000/XPI (DC11); Beckman Access (SF6); Siemens Centaur (CO10). The *B score* is the average bias of all Specimen % biases [(result – target)/target]×100% during the rolling 6-month window. The *C score* is the SD of the *B score* and shows consistency of bias over the same rolling time period. The *grey box* indicates the 5th to 95th centiles for each method. The *unfilled box* indicates the overall 5th to 95th centiles irrespective of method. The *dotted box* indicates limits of acceptable performance defined as ±20% *B score* and 20% *C score*. All analyses were performed during 2015. With permission from Birmingham Quality, University Hospitals Birmingham NHS Foundation Trust.

HOLOTRANSCOBALAMIN ASSAYS

Serum B₁₂ assays are unable to discriminate between haptocorrin-bound (referred to as holohaptocorrin) and transcobalamin-bound (holotranscobalamin) B₁₂. It is holotranscobalamin that is taken up via receptors to meet metabolic demand—holohaptocorrin and holotranscobalamin transport ~0.1 and 4.0 nmol/day of B₁₂ into cells, respectively.²³ The adoption of holotranscobalamin assays,^{24–26} to determine the abundance of the physiologically active form of cobalamin,²⁷ is increasingly widespread in Australia, Austria, Canada, Germany, Holland, Nordic countries, Switzerland and the UK. However, the mode of application is variable, that is, used as a sole status indicator,²⁸ as a first-line screening test in conjunction with a second-line test²⁴ and as a second-line test in conjunction with a serum B₁₂ assay.²⁹ Until recently, the automated ‘active B₁₂’ assay was only available using the Axis-Shield/Abbott Architect assay from Abbott. However, in February 2016, Siemens launched the Centaur Active-B₁₂ (AB12) assay for use outside of the USA.

Nexo *et al*³⁰ described a manual ELISA method permitting measurement of total transcobalamin and holotranscobalamin.²³ This technique has been used to demonstrate that ~10% of circulating transcobalamin is B₁₂-saturated with a reference range of 5–20%; and that 15–50% of B₁₂ is bound to transcobalamin. In B₁₂ deficiency, transcobalamin saturation falls to 0.4–3%.¹⁸

Utility as a screening test in mixed patient populations

A holotranscobalamin concentration <25 pmol/L strongly suggests B₁₂ deficiency. It is not possible to confidently exclude B₁₂ deficiency for holotranscobalamin results that fall in the range 25–70 pmol/L. Further investigation using a second-line test is necessary. An evaluation of the holotranscobalamin assay for the detection of B₁₂ deficiency against an MMA cut-off of 750 nmol/L showed sensitivities of ~65% and ~90% at 30 and 60 pmol/L, respectively. The corresponding assay specificity was ~90% and ~55%, respectively.¹²

Calibration

In response to the growing application of ‘active B₁₂’ assays, a consensus value for holotranscobalamin was assigned in October 2015 for the WHO International Standard 03/178.³¹

Physiological variation in holotranscobalamin concentration

There is more to learn about the determinants of holotranscobalamin concentration in the circulation, in particular a better understanding of the relative influences of B₁₂ metabolism and absorption is required.³² Clarity around which conditions, unrelated to B₁₂, influence holotranscobalamin also merits further investigation.

Analytical challenges associated with holotranscobalamin assays based on competitive binding luminescence technologies

Some patients carry a rare variant in the transcobalamin gene (TCN2) that interferes with the 'active B₁₂' assay.³³ The minor allele rs35838082 (p.R215W) is rare in Caucasians with a minor allele frequency (MAF) of <0.01 but more common in South Asians (MAF ~0.02) and those of African origin (MAF ~0.25). Holotranscobalamin results for these patients are erroneously low (~5 pmol/L) despite all other laboratory markers reported as normal, and an absence of clinical deficiency.³³

DIRECT COMPARISON OF THE HOLOTRANSCOBALAMIN AND SERUM B₁₂ ASSAYS

Direct comparison of the holotranscobalamin and serum B₁₂ assays is not straightforward because no single *gold standard* laboratory marker of vitamin B₁₂ status has been identified. However, receiver operator characteristic curves generated from a study in which subjects were defined as B₁₂ deficient if they had a serum MMA >750 nmol/L showed holotranscobalamin to be a moderately more reliable marker of B₁₂ status than serum B₁₂ based on a greater area under the curve, 0.85 vs 0.76 (B₁₂), and superior sensitivity and specificity.⁶

SECOND-LINE SCREENING OF VITAMIN B₁₂ STATUS

The inexorable link between assay sensitivity and specificity for both the serum B₁₂ and holotranscobalamin assays, and the variable clinical sequelae of B₁₂-deficient states leads to a wide indeterminate range. A serum B₁₂ concentration between 125 and 250 pmol/L and a holotranscobalamin concentration between 25 and 70 pmol/L merits investigation using functional laboratory markers that provide an indication of cellular B₁₂ utilisation.^{34 35}

Total homocysteine: an indicator of methylcobalamin-dependent methionine synthase function

In man, two different forms of B₁₂ (methylcobalamin and adenosylcobalamin) act as coenzymes for two different reactions.³⁵ In one, methionine synthase requires methylcobalamin for the remethylation of methionine from homocysteine. In the laboratory, tHcy is readily determined by a variety of automated analytical techniques. In countries where the addition of folic acid to all enriched cereal grain foods is mandated, an elevation in tHcy >20 µmol/L, with normal renal function, may indicate methionine synthase dysfunction in response to suboptimal B₁₂ (methylcobalamin form) availability.³⁶ Since cobalamin methylation, that is, optimal formation of methylcobalamin, is dependent on the supply of 5'-methyltetrahydrofolate, in countries without mandatory folic acid fortification the diagnostic utility of tHcy is limited unless it can be established that the patient is folate replete. 5'-Methyltetrahydrofolate is a more powerful nutritional determinant of homocysteine (~3.5 times) than methylcobalamin, and it is not until folate status has been optimised that B₁₂ becomes the major determinant of homocysteine.³⁷ Other, more modest, nutritional determinants of homocysteine include vitamin B₆ and vitamin B₂.³⁸ When interpreting tHcy results, it is recommended that age-specific and sex-specific reference ranges are applied. Examples from the author's laboratory include ≤13 and ≤15 µmol/L for females aged <60 years and males aged <64 years, respectively.³⁹

Methylmalonic acid: an indicator of adenosylcobalamin-dependent methylmalonyl-CoA mutase function

In the second of the two B₁₂-dependent reactions, methylmalonyl-CoA mutase uses adenosylcobalamin to convert methylmalonyl-CoA to succinyl-CoA. In B₁₂ deficiency, excess methylmalonyl-CoA is hydrolysed to MMA. MMA is a useful marker of B₁₂ utilisation, with some laboratories citing a serum concentration >280 nmol/L indicative of suboptimal status in patients <65 years with normal renal function.²⁴ Interpretation is more challenging in the elderly and those with impaired renal function.⁴⁰ An MMA concentration >750 nmol/L is generally accepted as indicative of 'definite' B₁₂ deficiency.³⁵ An automated LC-MS/MS assay for MMA analysis is available and capable of processing several hundred samples daily.⁴¹

MULTIASSAY APPROACHES TO THE LABORATORY DIAGNOSIS OF VITAMIN B₁₂ STATUS

Mathematical models that combine multiple markers of B₁₂ status into a single diagnostic indicator have been developed.⁴² The most sophisticated model uses all four most commonly available blood markers (a 'four-variable' analysis) to calculate a combined indicator of B₁₂ status (cB₁₂); where cB₁₂ is expressed as cB₁₂=log₁₀[(holotranscobalamin×serum B₁₂)/(methylmalonic acid×homocysteine)] (age factor). The formula transforms the four individual markers into a single variable dependent on age and is known as the 'Fedosov's wellness score'. cB₁₂ is interpreted as *high-normal*, *normal*, *low-normal*, *deficient* and *severely deficient*. 'Fedosov's wellness score' has recently been used to evaluate B₁₂ status in non-anaemic healthy Swiss senior citizens.⁴³

RECOMMENDED APPROACHES FOR THE LABORATORY ASSESSMENT OF B₁₂ STATUS

Although the application of a 'four-variable' analysis consisting of serum B₁₂, holotranscobalamin, tHcy and MMA to generate Fedosov's wellness score provides a rigorous approach for the laboratory evaluation of B₁₂ status, cost precludes its routine application. 'Three-variable' or 'two-variable' analysis approaches have also been evaluated and shown to estimate B₁₂ status within acceptable margins of error compared with the 'four-variable' analysis.⁴⁵

Recommended 'three-variable' analysis: serum B₁₂, holotranscobalamin and MMA

Using models developed that calculate the Fedosov's wellness score based on the application of three assays being used in combination with a correction for folate on the model through its modulation of homocysteine,⁴⁵ the best simulation of the 'four-variable' analysis is achieved through the combination of assays for serum B₁₂, holotranscobalamin and MMA. tHcy should be omitted.

The next best 'three-variable' analysis is achieved by omitting either serum B₁₂ or holotranscobalamin from the 'four-variable' analysis and combining the chosen assay with MMA and tHcy.⁴⁵

Recommended 'two-variable' analysis: holotranscobalamin and MMA

The smallest 'two-variable' analysis error (compared with the 'four-variable' analysis) is observed when holotranscobalamin is combined with MMA.⁴⁵ It is this approach that the author recommends for laboratory diagnosis of B₁₂ status since an

Best practice

acceptable deviation from the 'four-variable' analysis is achieved without the expense of performing additional tests.

EXAMPLE LABORATORY ASSESSMENT ALGORITHM

An example laboratory assessment algorithm that has been used extensively in the author's laboratory since 2012 that uses holotranscobalamin (first-line assay) with MMA (second-line assay) is shown in figure 2A. In laboratories where holotranscobalamin is not available, the recommended approach is serum B₁₂ combined with MMA (figure 2A). Note that in the interest of economy in this example a sequential selection algorithm is followed, that is, whether a second-line assay is performed is dependent on the outcome of the first-line assay rather than both tests being performed on all samples. In the author's laboratory, ~5% of samples from a mixed patient population have a holotranscobalamin <25 pmol/L and are classified as deficient; an indeterminate concentration of 25–70 pmol/L is measured in ~25% of samples leading to second-line MMA analysis. All other samples are classified as replete. Figure 2B

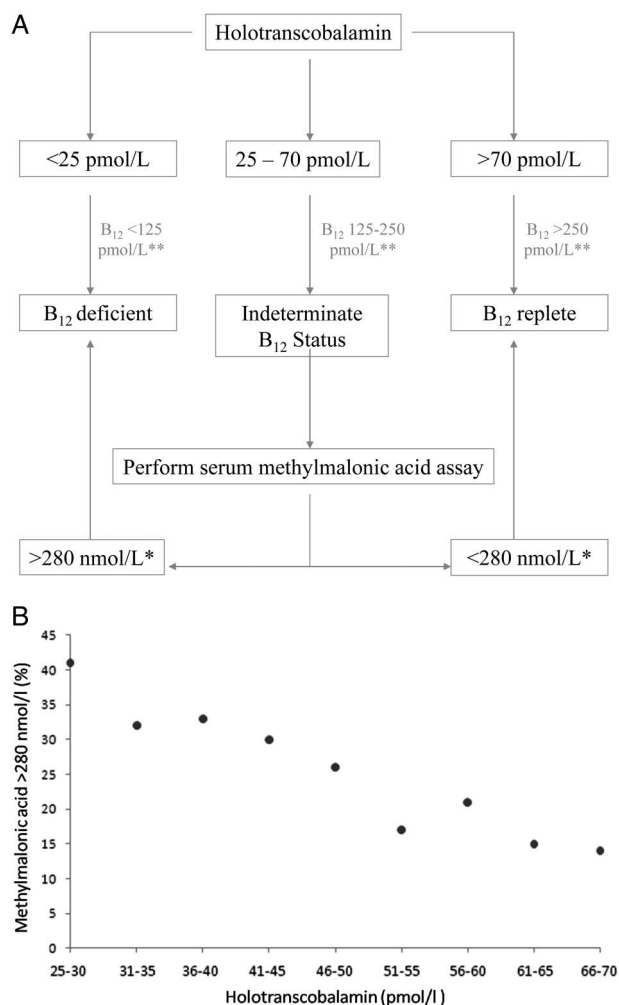


Figure 2 (A) An example laboratory assessment algorithm for vitamin B₁₂ status assessment that uses holotranscobalamin and methylmalonic acid. *Cut-off applied to patients <65 years old with good renal function (a cut-off of >360 nmol/L applied to patients >65 years). **Previously suggested cut-offs for serum B₁₂ from Hvas and Nexo.³⁴ (B) Percentage of patients with a methylmalonic acid concentration >280 nmol/L as a function of holotranscobalamin concentration in a mixed patient population (n=3122).^{24 46} *A cut-off of >360 nmol/L applied to patients >65 years.

shows the percentage of samples from the indeterminate range subsequently defined as B₁₂ deficient. Total prevalence of B₁₂ deficiency in the mixed patient population is 10.8%.²⁴

IDENTIFYING THE AETIOLOGY OF VITAMIN B₁₂ DEFICIENCY

Schilling test

The withdrawal of radiolabelled cyanocobalamin and bovine intrinsic factor reagents for the Schilling test hampers the investigation of patients with B₁₂ deficiency. The test consisted of up to four parts (part I, basic test; part II, with intrinsic factor; part III, following course of antibiotics; part IV, pancreatic enzymes taken for 3 days) in which the urinary excretion of radiolabelled B₁₂ with and without intrinsic factor was established.^{47 48} Result interpretation was as follows: part I <5% labelled B₁₂ excreted and part II excretion normal or near normal confirmed malabsorption as a result of lack of intrinsic factor (eg, pernicious anaemia); parts I and II abnormal, suggested malabsorption not resulting from intrinsic factor deficiency, for example, Crohn's disease; part III abnormal indicated abnormal bacterial growth. Part IV abnormal indicated pancreatic insufficiency.

As a sensitive marker of B₁₂ malabsorption, holotranscobalamin levels that correct with small oral doses of vitamin B₁₂ and the use of recombinant intrinsic factor could provide the basis for a non-isotopic B₁₂ absorption test to replace the unavailable Schilling test. A sensitive B₁₂ absorption test described by Nexo *et al* that relies on the holotranscobalamin assays (CobaSorb) to identify which patients may benefit from oral courses of B₁₂ rather than the more commonly used replacement by intramuscular injection is available.⁴⁹ The CobaSorb test involves measuring holotranscobalamin before and two days after daily intake of three times 9 µg B₁₂ (cyanocobalamin form). The authors assigned a cut-off of >22% and >10 pmol/L to demonstrate active absorption with the caveat that it should not be used if the baseline B₁₂ level is >65 pmol/L—in this situation, the C-CobaSorb test is suggested.⁵⁰

Other laboratory assays that can be used to establish the cause of B₁₂ deficiency include those for plasma-intrinsic factor antibodies; plasma-gastrin and pepsinogen; and plasma-parietal cell antibodies.

Two types of plasma-intrinsic factor antibodies have been detected in the plasma of >60% of patients with pernicious anaemia^{44 51} with type I blocking the binding of B₁₂ to intrinsic factor and type II stopping the attachment of intrinsic factor or intrinsic factor-B₁₂ complex to ileal receptors. Achlorhydria may be suspected by the presence of raised gastrin levels.⁵²

Ninety per cent of patients with pernicious anaemia have gastric parietal cell antibodies, but specificity of this test is poor since they are also found in 15% of elderly subjects.

SUMMARY

No single laboratory marker is suitable for the assessment of B₁₂ status in all patients. The application of multiple markers, whether using a sequential selection algorithm or through the calculation of a single diagnostic indicator such as cB₁₂, leads to a reduction in the number of B₁₂-deficient patients who are overlooked. In the presence of discordance between laboratory test result and strong clinical features of B₁₂ deficiency, it remains important to proceed with treatment to avoid neurological impairment.⁵

Handling editor Tahir Pillay

Twitter Follow Dominic Harrington at @DrDJHarrington

Acknowledgements The author thanks Finlay MacKenzie and Aimee McGrory from UK NEQAS Haematinics for providing figure 1. The UK NEQAS Haematinics service is provided by Birmingham Quality, which is part of the University Hospitals Birmingham NHS Foundation Trust. The author is particularly indebted to Dr Malcolm Hamilton, Anne Lee and Sheena Blackmore for sharing their expertise. Thanks to Dr Agata Sobczyńska-Malefora for her work in establishing age-dependent and sex-dependent reference range for tHcy, Renata Gorska for assistance with the preparation of this manuscript, and to the wider team from the Nutristasis Unit at Guy's and St. Thomas' Hospital NHS Foundation Trust for the ongoing development and application of assays to establish vitamin status.

Competing interests None declared.

Provenance and peer review Commissioned; externally peer reviewed.

REFERENCES

- 1 Stabler SP. Clinical practice. Vitamin B₁₂ deficiency. *N Engl J Med* 2013;368:149–60.
- 2 Hunt A, Harrington D, Robinson S. Vitamin B12 deficiency. *BMJ* 2014;349:5226.
- 3 Lindenbaum J, Healton EB, Savage DG, et al. Neuropsychiatric disorders caused by cobalamin deficiency in the absence of anemia or macrocytosis. *N Engl J Med* 1988;318:1720–8.
- 4 Hvas AM, Ellegaard J, Nexø E. Increased plasma methylmalonic acid level does not predict clinical manifestations of vitamin B12 deficiency. *Arch Intern Med* 2001;161:1534–41.
- 5 Devalia V, Hamilton MS, Molloy AM., British Committee for Standards in Haematology. Guidelines for the diagnosis and treatment of cobalamin and folate disorders. *Br J Haematol* 2014;166:496–513.
- 6 Vakur Bor M, Nexø E. Vitamin B12—cobalamin. In: Herrman W, Obeid R, eds. *Vitamins in the prevention of human diseases*. Berlin: De Gruyter, 2011:187–99.
- 7 Harmening DM. *Megaloblastic anemia: clinical haematology and fundamentals of haemostasis*. F A Davis Company, 2001:112–19.
- 8 Provan D, Singer C, Baglin T, et al. Red cell disorders. In: *Oxford handbook of clinical haematology*. 3rd edn. Oxford University Press, 2010:46–47.
- 9 Hamilton MS, Blackmore S. Investigation of megaloblastic anaemia cobalamin, folate and metabolite status. In: Bain BJ, Bates I, Laffan MA, eds. *Dacie and Lewis practical haematology*. 11th edn. Churchill Livingstone, 2012:202–24.
- 10 Lindenbaum J, Nath BJ. Megaloblastic anaemia and neutrophil hypersegmentation. *Br J Haematol* 1980;44:511–13.
- 11 Healton EB, Savage DG, Brust JC, et al. Neurologic aspects of cobalamin deficiency. *Medicine (Baltimore)* 1991;70:229–45.
- 12 Clarke R, Sherliker P, Hin H, et al. Detection of vitamin B12 deficiency in older people by measuring vitamin B12 or the active fraction of vitamin B12, holotranscobalamin. *Clin Chem* 2007;53:963–70.
- 13 Snow CF. Laboratory diagnosis of vitamin B12 and folate deficiency: a guide for the primary care physician. *Arch Intern Med* 1999;159:1289–98.
- 14 Herrmann W, Obeid R, Schorr H, et al. The usefulness of holotranscobalamin in predicting vitamin B12 status in different clinical settings. *Curr Drug Metab* 2005;6:47–53.
- 15 Morkbak AL, Hvas AM, Milman N, et al. Holotranscobalamin remains unchanged during pregnancy. Longitudinal changes of cobalamins and their binding proteins during pregnancy and post partum. *Haematologica* 2007;92:1711–12.
- 16 Maniati V, Davidson BR, Rolles K, et al. Fibrolamellar hepatocellular carcinoma: prolonged survival with multimodality therapy. *Eur J Surg Oncol* 2009;35:617–21.
- 17 Jacob E, Herbert V. Measurement of unsaturated “granulocyte-related” (TC I and TC III) and “liver-related” (TC II) B12 binders by instant batch separation using a microfine precipitate of silica (QUSO G32). *J Lab Clin Med* 1975;86:505–12.
- 18 Nexø E, Christensen A, Hvas A, et al. Quantification of holotranscobalamin, a marker of vitamin B12 deficiency. *Clin Chem* 2002;48:561–2.
- 19 Scott JM, Bloomfield FJ, Stebbins R, et al. Studies on derivation of transcobalamin 3 from granulocytes: enhancement by lithium and elimination by fluoride of in vitro increments in vitamin B₁₂-binding capacity. *J Clin Invest* 1974;53:228–39.
- 20 Chanarin I. *The megaloblastic anaemias*. 3rd edn. London: Blackwell Scientific Publications, 1990.
- 21 Carmel R, Agrawal YP. Failures of cobalamin assays in pernicious anemia. *N Engl J Med* 2012;367:385–6.
- 22 Thorpe SJ, Heath AS, Blackmore S, et al. International standard for serum B₁₂ and serum folate: international collaborative study to evaluate a batch of lyophilised serum for B₁₂ and folate content. *Clin Chem Lab Med* 2007;45:380–6.
- 23 Nexø E, Hoffmann-Lücke E. Holotranscobalamin, a marker of vitamin B-12 status: analytical aspects and clinical utility. *Am J Clin Nutr* 2011;94:359S–65S.
- 24 Sobczyńska-Malefora A, Gorska R, Pelisser M, et al. An audit of holotranscobalamin (‘Active’ B₁₂) and methylmalonic acid assays for the assessment of vitamin B₁₂ status: application in a mixed patient population. *Clin Biochem* 2014;47:82–6.
- 25 Ulleland M, Eilertsen I, Quadros EV, et al. Direct assay for cobalamin bound to transcobalamin (holotranscobalamin) in serum. *Clin Chem* 2002;48:526–32.
- 26 Brady J, Wilson L, McGregor L, et al. Active B₁₂: a rapid automated assay for holotranscobalamin on the Abbott AxSYM analyser. *Clin Chem* 2008;54:567–73.
- 27 Herzlich B, Herbert V. Depletion of serum holotranscobalamin. II. An early sign of negative vitamin B₁₂ balance. *Lab Invest* 1988;58:332–7.
- 28 Heil SG, de Jonge R, de Rotte MC, et al. Screening for metabolic vitamin B12 deficiency by holotranscobalamin in patients suspected of vitamin B12 deficiency: a multicentre study. *Ann Clin Biochem* 2012;49:184–9.
- 29 Miller JW, Garrod MG, Rockwood AL, et al. Measurement of total vitamin B12 and holotranscobalamin, singly and in combination, in screening for metabolic vitamin B12 deficiency. *Clin Chem* 2006;52:278–85.
- 30 Nexø E, Christensen AL, Petersen TE, et al. Measurement of transcobalamin by ELISA. *Clin Chem* 2000;46:1643–9.
- 31 Thorpe SJ, Rigsby P, Roberts G, et al. An International Standard for holotranscobalamin (holoTC): international collaborative study to assign a holoTC value to the International Standard for vitamin B12 and serum folate. *Clin Chem Lab Med* 2016.
- 32 Carmel R. On measuring and interpreting holotranscobalamin (holotranscobalamin II) [Editorial]. *Clin Chem* 2002;48:407–9.
- 33 Sobczyńska-Malefora A, Pangilinan FJ, Plant GT, et al. Association of a transcobalamin II genetic variant with falsely low results for the holotranscobalamin immunoassay. *Eur J Clin Invest* 2016;46:434–9.
- 34 Hvas AM, Nexø E. Diagnosis and treatment of vitamin B12 deficiency—an update. *Haematologica* 2006;91:1506–12.
- 35 Obeid R, Herrmann W, Vitamin B₁₂—cobalamin. In: Herrmann W, Obeid R, eds. *Vitamins in the prevention of human diseases*. De Gruyter: Berlin, 2011:187–271.
- 36 Clarke R, Grimley Evans J, Schneede J, et al. Vitamin B12 and folate deficiency in later life. *Age Ageing* 2004;33:34–41.
- 37 Quinlivan BP, McPartlin J, McNulty H, et al. Importance of both folic acid and vitamin B12 in reduction of risk of vascular disease. *Lancet* 2002;359:227–8.
- 38 Refsum H, Smith AD, Ueland PM, et al. Facts and recommendations about total homocysteine determinations: an expert opinion. *Clin Chem* 2004;50:3–32.
- 39 Sobczyńska-Malefora A, Harrington DJ, Voong K, et al. Plasma and red cell reference intervals of 5-methyltetrahydrofolate of healthy adults in whom biochemical functional deficiencies of folate and vitamin B₁₂ had been excluded. *Adv Hematol* 2014;2014:465623.
- 40 Vogiatzoglou A, Oulhaj A, Smith AD, et al. Determinants of plasma methylmalonic acid in a large population: implications for assessment of vitamin B12 status. *Clin Chem* 2009;55:2198–206.
- 41 Gorska R, Harrington DJ, Roberts P. Development of a fully automated method for determination of methylmalonic acid in human serum/plasma by MPS-LC-MS/MS [abstract]. *CCLM* 2014;52:eA311.
- 42 Fedosov SN. Metabolic signs of vitamin B(12) deficiency in humans: computational model and its implications for diagnostics. *Metab Clin Exp* 2010;59:1124–38.
- 43 Risch M, Meier DW, Sakem B, et al. Vitamin B12 and folate levels in healthy Swiss senior citizens: a prospective study evaluating reference intervals and decision limits. *BMC Geriatr* 2015;15:82.
- 44 Shackleton PJ, Fish DI, Dawson DW. Intrinsic factor antibody tests. *J Clin Pathol* 1989;42:210–2.
- 45 Fedosov SN, Brito A, Miller JW, et al. Combined indicator of vitamin B12 status: modification for missing biomarkers and folate status and recommendations for revised cut-points. *Clin Chem Lab Med* 2015;53:1215–25.
- 46 Sobczyńska-Malefora A, Critcher MS, Harrington DJ. The application of holotranscobalamin and methylmalonic acid in hospital patients and total vitamin B₁₂ in primary care patients to assess low vitamin B₁₂ status. *J Hematol Thromb* 2015;1:8–16.
- 47 Schilling RF. Intrinsic factor studies. II. The effect of gastric juice on the urinary excretion of radioactivity after the oral administration of radioactive vitamin B₁₂. *J Lab Clin Med* 1953;42:860–6.
- 48 International Committee for Standardization in Haematology. Recommended method for the measurement of vitamin B₁₂ absorption. *J Nucl Med* 1981;22:1091–3.
- 49 Bhat DS, Thuse NV, Lubree HG, et al. Increases in plasma holotranscobalamin can be used to assess vitamin B-12 absorption in individuals with low plasma vitamin B-12. *J Nutr* 2009;139:2119–23.
- 50 Hardlei TF, Morkbak AL, Bor MV, et al. Assessment of vitamin B(12) absorption based on the accumulation of orally administered cyanocobalamin on transcobalamin. *Clin Chem* 2010;56:432–6.
- 51 Waters HM, Smith C, Howarth JE, et al. A new enzyme immunoassay for the detection of total type I and type II intrinsic factor antibody. *J Clin Pathol* 1989;42:307–12.
- 52 Slingerland DW, Cararelli JA, Burrows BA, et al. The utility of serum gastrin levels in assessing the significance of low serum B₁₂ levels. *Arch Intern Med* 1984;144:1167–8.



Laboratory assessment of vitamin B₁₂ status

Dominic J Harrington

J Clin Pathol 2017 70: 168-173 originally published online May 11, 2016
doi: 10.1136/jclinpath-2015-203502

Updated information and services can be found at:
<http://jcp.bmj.com/content/70/2/168>

| | |
|-------------------------------|--|
| | <i>These include:</i> |
| References | This article cites 45 articles, 17 of which you can access for free at: http://jcp.bmj.com/content/70/2/168#BIBL |
| Email alerting service | Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article. |

| | |
|--------------------------|--|
| Topic Collections | Articles on similar topics can be found in the following collections Editor's choice (137) JCP Education (167) |
|--------------------------|--|

Notes

To request permissions go to:
<http://group.bmj.com/group/rights-licensing/permissions>

To order reprints go to:
<http://journals.bmj.com/cgi/reprintform>

To subscribe to BMJ go to:
<http://group.bmj.com/subscribe/>