Editorial

Dominic J. Harrington

Holotranscobalamin: in the middle of difficultly lies opportunity

The authors of this article [1] in this issue of Clinical Chemistry and Laboratory Medicine have made the adoption of holotranscobalamin for the laboratory assessment of vitamin B₁₂ (B₁₂) status a more attractive proposition. They have achieved this by undertaking an international collaborative study to assign a holotranscobalamin value to the World Health Organisation (WHO) International Standard (IS) 03/178 for vitamin B₁₂ and folate. Endorsement of the assigned consensus value by the WHO Expert Committee on Biological Standardization is timely and important because the availability of an IS offers the opportunity to facilitate further development of assay methodologies for the determination of holotranscobalamin, and supports the meaningful comparison of population data.

An IS for holotranscobalamin is the latest landmark in the difficult search for a reliable and convenient laboratory marker of B₁₂ status. For two decades, Cohn, Minot and collaborators were wholly dependent on laborious bioassays to isolate the uncharacterised liver constituent that would be an effective treatment for patients with pernicious anaemia [2]. This reliance endured until 1949 when Hutner et al. successfully exploited the B₁₂-dependency of Euglena gracilis, a chlorophyl-containing protozoon found in pond water, to develop a microbiological assay for the antianaemic factor [3]. Ross subsequently modified the assay for the determination of B₁₂ (as the antianaemic factor became known) in body fluids [4]. Microbiological assays for B₁₂ proved to be both sensitive and specific, but intrinsically time consuming.

Estimations of B₁₂ status were made more quickly from 1961, thanks to the application of the first of many radioisotope-based dilution assays to be described [5]. Unfortunately, disparities between results generated by microbiological and radioisotope assays of B₁₂ were not uncommon. From the beginning of the 1990s, the abundance of B₁₂ in serum could be determined using highly automated competitive-binding luminescence based assays (CBLA), and it is this approach that predominates in clinical laboratories today. CBLA-based methods are rapid and well suited to satisfying the current high demand for B₁₂ analysis – but failures of assays based on this technology have been documented and include a propensity to generate erroneous results in some patients with previously proven pernicious anaemia [6].

Other markers of B₁₂ status are widely available. These include the concentration of methylnalonic acid and total homocysteine in serum, which are known to partly reflect tissue utilisation of adenosylcobalamin and methylcobalamin, respectively. The theoretical merits of measuring holotranscobalamin for the assessment of B₁₂ status have also been long debated. The attractiveness of holotranscobalamin rests on the fact that cellular uptake of cobalamin is reliant on a receptor mediated endocytosis process involving transcobalamin, a plasma protein that carries cobalamin and a cell surface receptor that specifically binds cobalamin saturated transcobalamin. Only a minor fraction of circulating cobalamin measured by serum B₁₂ assays is bound to transcobalamin. It is this fraction that is known as holotranscobalamin. The availability of at least four distinct laboratory markers has not deterred the majority of laboratories from exclusively using the total abundance of B₁₂ in serum as a sole indicator of status. However, it is increasingly accepted that it is not possible to confidently exclude B₁₂ deficiency in all patients using any one single marker in isolation [7]. Alongside this recognition is a shift in laboratory practice that has seen the use of holotranscobalamin increasingly widespread in Australia, Austria, Canada, Germany, Holland, Nordic countries, Switzerland and the UK.

The increased application of holotranscobalamin follows a period during which the clinical utility of this laboratory marker had remained largely untested through the lack of a suitable analytical method. This barrier began to lift when an expert group published a method in 2002 that combined a sensitive enzyme-linked immunosorbent assay (ELISA) method for transcobalamin with a procedure for the removal of transcobalamin not carrying B₁₂ [8]. The group also collaborated with the development of commercial assays for holotranscobalamin, with the first of two iterations of radioimmunoassay from Axis-Shield.
released at the beginning of 2001 (the commercial radio-immunoassay was withdrawn in 2007). An automated random-access analyser method for holotranscobalamin (marketed as ‘active B12’) on the Abbott AxSYM analyser followed (worldwide launch excluding USA 27th June 2006) [9], which in turn was superseded by an assay on the Abbott Architect, a two-step quantitative immunoassay using chemiluminescent microparticle immunoassay technology (27th September 2011). An ELISA assay from Axis-Shield was the next to launch (13th April 2012) and more recently Siemens released the Centaur Active-b12 (AB12) assay for use outside of the United States (22nd January 2016). The Biohit Active B12 ELISA and IBL International Active-B12 (holotranscobalamin) ELISA are also available. Emerging evidence from the application of these methods indicates that holotranscobalamin is a more reliable marker of B12 status than serum B12 assays. Yet this view is not one that is universally held [10, 11], and it is widely acknowledged that there is much more to learn. For example, two groups have recently reported rare variants in the transcobalamin gene that interfere with the ‘active B12’ assay [12, 13] – a clear reminder that no assay for the assessment of B12 is without biological or technical limitation.

Although there is little that can be done to entirely mitigate biological factors that continue to bring so much uncertainty to the laboratory assessment of B12 status, improved standardisation of the assays themselves is addressable. After all, regardless of the chosen marker, the correct assessment of B12 status is reliant on the accuracy of results generated by clinical laboratories. Surprisingly, standardisation has never been achieved for the measurement of total B12 in serum. Notable missed opportunities for standardisation began in the 1980s when seven laboratories participated in a collaborative study using E. gracilis as the test organism in the turbidimetric Euglena B12 assay, for the analysis of an amplified preparation of normal serum labelled 81/563. As a result, in 1985, the National Biological Standards Board established the preparation as the British Standard for Human Serum Vitamin B12 and assigned it a potency of 320 pg/mL [14]. In 1992, this standard was reclassified as the first WHO IS, (81/563). However, the preparation was found to be positive for anti-HCV and HCV RNA, and having been calibrated by microbiological assays, which in essence reflect B12 function rather than abundance as determined by automated analytical platforms, it was decided to replace the first WHO IS and assign a value to a replacement preparation using contemporary methodology. Twenty-three laboratories in seven countries analysed pooled human serum from seven donors donated by the UK NEQAS Scheme for Haematinics and assigned a potency of 480 pg to the second WHO IS for serum B12 (03/178) that was ratified in 2007 [15]. Reassuringly, the simultaneous revaluation of the first WHO IS for human serum vitamin B12 (81/563) was 332 pg/mL (103.75% of the value assigned in 1985).

It is unfortunate then that the first and second WHO IS for B12, and folate have been little used. There are several possible explanations for the poor uptake. One being that for an IS to be of the highest metrological level of traceability the reference material should be calibrated in SI units using a primary reference measurement procedure. Unfortunately, this is not easily achieved for B12 because of the multiple forms of the vitamin that are present at a range of proportions. A reference analytical method for B12 is also lacking.

To this day, serum B12 assays still tend to be calibrated independently by manufacturers with traceability to an internally manufactured standard material. Predictably, this has led to poor agreement between commercially available assays for serum B12 as evidenced by UN NEQAS survey returns [7]. Concepts of measurement accuracy and standardisation have progressed since the genesis of today’s serum B12 assays. This has been brought into sharp focus by the introduction of an internationally recognised standard that details the requirements for quality and competence expected of medical laboratories (ISO 15189:2012). ISO 15189:2012 affirms the need for metrological traceability to a reference material or reference procedure of the higher metrological order available.

At present all commercially available assays for holotranscobalamin use distinct calibrators that are traceable back to common frozen primary reference calibrators which are held by Axis-Shield. The reference calibrators were prepared gravimetrically from a stock solution of recombinant human holotranscobalamin and adjusted using a panel of serum samples of ‘known’ holotranscobalamin concentration to correct for the difference in ionic strength and pH between the calibrator buffer and serum samples (the ‘known’ values having been assigned by the RIA assay which was the predicate device for subsequent assays). Each batch of calibrators that is released to the market is matched against this material. Now that a value for holotranscobalamin has been added to IS 03/178, Axis-Shield are considering how this material may be used to maintain assay calibration and further aid assay alignment. However, this is rather circular since the calibrators used to assign the holotranscobalamin value to IS 03/178 overwhelmingly originate from Axis-Shield.

In the coming years, we will continue to understand more of the merits and caveats associated with the holotranscobalamin assay for the assessment of B12 status.
With its increased application in large patient populations will come acceleration in this learning – a process that it is hoped will be aided by early holotranscobalamin assay harmonisation.

**Author contributions:** The author has accepted responsibility for the entire content of this submitted manuscript and approved submission.

**Research funding:** None declared.

**Employment or leadership:** None declared.

**Honorarium:** None declared.

**Competing interests:** The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

**References**


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, Phone: +44 (0) 207 188 6816, E-mail: Dominic.Harrington@Viapath.co.uk; and Division of Women’s Health, School of Medicine, King’s College London, London, UK.