AUSTRALIAN Clinicallabs

GP

Will the Real Assessment of Vitamin B12 Deficiency Please Stand Up?

By Associate Professor Chris Barnes

The MBS changes introduced in 2025 have prompted necessary reflection on how vitamin B12 deficiency is best assessed in clinical practice. Our laboratory previously published an article outlining the potential advantages of Active B12 testing and its role in detecting early deficiency. With the MBS review in June 2025, it is timely to revisit that position, acknowledge the limitations of holoTC, and consider alternative strategies that prioritise the detection of functional B12 deficiency. Functional vitamin B12 deficiency refers to impaired intracellular cobalamin activity despite normal or borderline serum B12 or holo-transcobalamin levels (Active B12), leading to accumulation of the metabolic intermediates methylmalonic acid (MMA) and homocysteine. In the context of the 2025 MBS changes, this biochemical phenotype may be appropriately detected through reflex testing.

Revisiting the complexity of B12 deficiency

Vitamin B12 deficiency remains challenging because no single test can accurately diagnose or exclude deficiency in ***all*** clinical settings. Total serum B12 lacks sensitivity, and clinically meaningful (functional) deficiency can occur even when levels fall within the laboratory reference range.[1] This is particularly relevant in older adults, where cognitive decline, neuropathy, anaemia, and frailty may develop subtly and be mistaken for ageing.[2]

Risk factors for the development of vitamin B12 deficiency are common and often cumulative, including:

- Long-term proton pump inhibitor or H2-blocker therapy
- Metformin use
- Pernicious anaemia or autoimmune gastritis
- Older age, gastric atrophy and reduced intrinsic factor production[3]

Vitamin B12 deficiency remains challenging because no single test can accurately diagnose or exclude deficiency in all clinical settings.

Active B12: useful but far from perfect

Although Active B12 reflects the biologically available fraction of cobalamin, several practical and biological limitations should be acknowledged.

- Both serum vitamin B12 and holo-transcobalamin testing are characterised by a wide diagnostic “grey zone” that frequently necessitates confirmatory investigations and are significantly influenced by binding-protein biology.
- Active B12 measures cobalamin bound to transcobalamin II, the carrier protein responsible for cellular delivery, rather than serving as a direct marker of intracellular B12 activity.
- Transcobalamin II concentrations and receptor function are influenced by inflammation, liver and renal disease, ageing and genetic variation, such that holo-transcobalamin may appear normal or elevated despite impaired cellular utilisation, with documented cases demonstrating markedly elevated methylmalonic acid and homocysteine in the presence of normal serum and Active B12 levels.

A functional, clinically aligned pathway: reflex HCY testing

As methylmalonic acid is technically available under the MBS but requires specialised mass-spectrometry platforms and is not routinely accessible in many laboratories, and in response to the recent MBS changes aimed at improving identification of clinically significant (functional) vitamin B12 deficiency, our laboratory will implement reflex homocysteine (HCY) testing for all serum vitamin B12 results below 360 pmol/L, reframing this interval as "borderline" rather than "low-normal."

This approach ensures that individuals at risk of early or functional deficiency, particularly older patients, receive additional biochemical clarification without the need for a separate test request or delays associated with referral MMA testing.

However, meaningful interpretation of HCY is essential:

- Mild elevations (16–20 µmol/L) are often non-specific and may reflect renal function, folate or B6 deficiency, or pre-analytical factors
- HCY >20 µmol/L correlates more strongly with clinically significant B12 deficiency and aligns with historic Active B12–based detection thresholds [1,6]

Reports will now include the updated interpretive comment:

"HCY is above the laboratory reference range, but values below 20 µmol/L are often non-specific and may not indicate clinically significant B12 deficiency. Levels >20 µmol/L are more consistent with vitamin B12 deficiency."

> *Our laboratory will now implement reflex homocysteine (HCY) testing for all serum B12 results below 360 pmol/L, reframing this interval as "borderline" rather than "low-normal."*

Where does this leave GPs?

The inevitable question is: what is the real measure of B12 deficiency?

In practice, there is no single biochemical marker that answers this confidently. Clinical context, risk factors, and functional testing provide the most reliable pathway forward.

Our updated approach aims to:

- Avoid missed deficiency in high-risk groups
- Reduce over-calling of non-specific biochemical abnormalities
- Provide clearer, more clinically aligned interpretive comments
- Support GPs in making confident, evidence-based decisions in assessing patients at risk of vitamin B12 deficiency

---

About the author:

Assoc. Prof. Chris Barnes

MBBS FRACP FRCPA

Lab: Clayton (Vic)
Speciality: Haematology
Areas of Interest: Paediatric haematology, non-malignant haematological conditions including thrombosis and bleeding disorders
Phone: (03) 9538 6777
Email: chris.barnes@clinicallabs.com.au

References

1. Hunt A, Harrington D, Robinson S. Vitamin B12 deficiency. *BMJ*. 2014.
2. Wolters M *et al.* Cobalamin in the elderly. *Prev Med.* 2004.
3. O'Leary F, Samman S. Vitamin B12 in health and disease. *Nutrients*. 2010.
4. Russell-Jones G. *ASEAN J Psychiatry*. 2022.
5. Russell-Jones G. Paradoxical Vitamin B12 Deficiency. 2023.
6. Stabler SP. Vitamin B12 deficiency. *N Engl J Med*. 2013.

ACLMAR-BF-NAT-0847.3 02/26 ACLZLFT0121