



ACTIVE-B12 **EIA**

the next level of B12 testing



Vitamin B12 – an essential nutrient

Vitamin B12 is an essential nutrient (can only be obtained from the diet) and is a vital component in many cellular functions including DNA synthesis. Deficiency can result in cognitive problems, dizziness and numbness and if left untreated, irreversible neurological damage.

Vitamin B12 deficiency is a major public health issue and is widespread. Estimates suggest that up to 20% of the elderly population may be deficient or deplete in Vitamin B12¹ and with an increasingly ageing global population the situation will only worsen.

Among other population groups at risk are pregnant women and their offspring, vegetarians, and patients with intestinal diseases or those taking acid suppressants.

Left untreated, B12 deficiency can result in irreversible neurological damage. Early and accurate detection of Vitamin B12 deficiency is therefore vital.

Diagnosis of B12 deficiency is notoriously difficult; the classic haematological signs and symptoms may be absent or non-specific and currently-used laboratory methods can give misleading results.

The Active-B12 EIA is an innovative new method for assessing Vitamin B12 status and offers improved accuracy, sensitivity and specificity over current laboratory methods.



What is Active-B12?

Three carrier proteins are involved in the transport of Vitamin B12 around the body - Intrinsic Factor (IF), transcobalamin (TC) and haptocorrin (HC).

When transcobalamin and haptocorrin bind Vitamin B12 the resulting complexes are known as holotranscobalamin (HoloTC) and holohaptocorrin (HoloHC).

HoloTC represents only 10-30% of the Vitamin B12 circulating in the blood but is the **ONLY** form of Vitamin B12 that is taken up and used by cells of the body, hence it's other name... **ACTIVE-B12**.

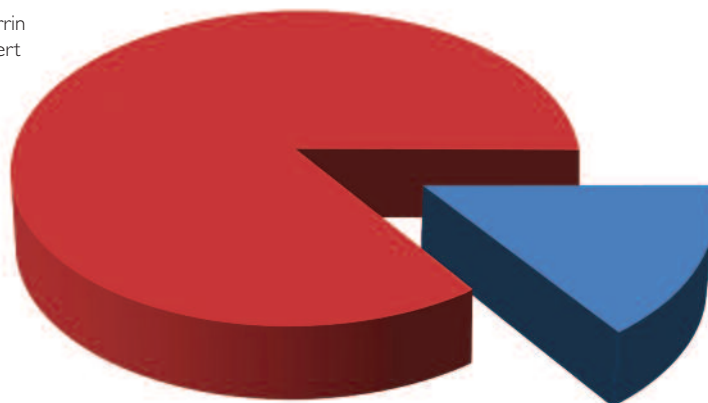
Only transcobalamin transports Vitamin B12 from its site of absorption in the ileum to tissues and cells. The vitamin is then internalised as the Active-B12 complex via a specific receptor-mediated uptake. This process delivers Vitamin B12 into the cells of the body and provides the vitamin as an essential co-enzyme for vital cellular functions such as DNA synthesis.

The remaining 70-90% of circulating Vitamin B12 is bound to haptocorrin, the function of which is unknown.

Also, as Active-B12 has a shorter circulating half-life compared to holohaptocorrin, the earliest change that occurs on entering negative vitamin B12 balance is very likely to be a decrease in Active-B12 concentration⁸.

Vitamin B12 in the circulation

Holohaptocorrin
Biologically inert
70-90%



Active-B12
(Holotranscobalamin)
Biologically active
10-30%



Measuring B12

The current front-line test for assessing Vitamin B12 status is the total B12 assay but this test has a number of important limitations:

False positives

A proportion of individuals with Total B12 levels below the common cut-off of 148pmol/L (200pg/mL) show no clinical or biochemical evidence of deficiency².

False negatives

Conversely, neuropsychiatric and metabolic abnormalities can occur with total B12 concentrations well within the normal reference interval^{2,3}.

Measures all circulating Vitamin B12

But not all B12 in the blood is available to be used by the body. Only B12 bound to transcobalamin, Active-B12, is taken up from the blood into the cells of the body.

Large proportion of indeterminate results

There are many publications attesting to a significant indeterminate zone when using the Total B12 assay^{4,5,6}

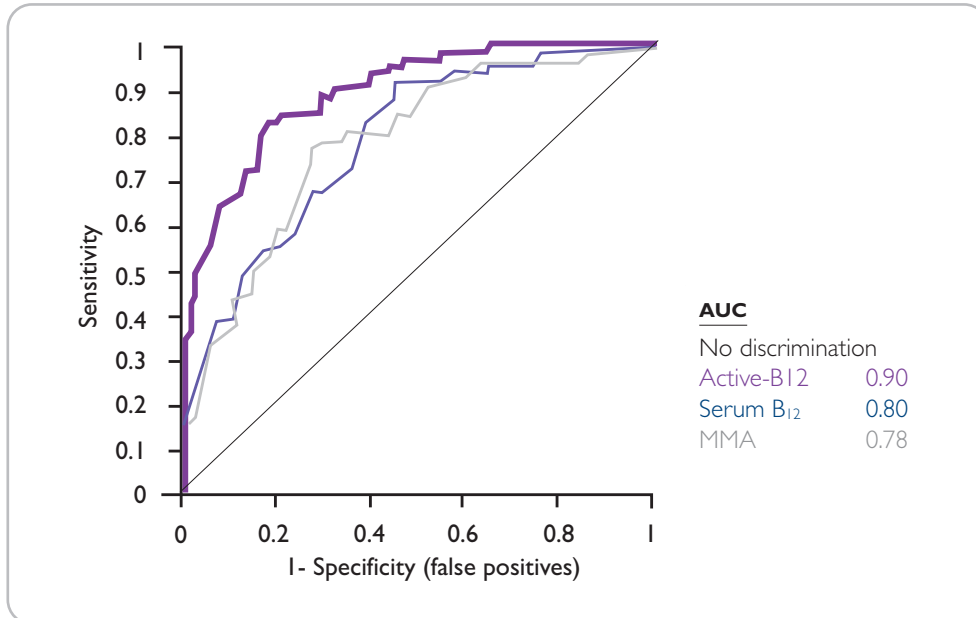
It is estimated that approximately half of patients with B12 deficiency are not detected when the Total B12 test is used with a cut-off of 148pmol/L (200pg/mL)⁷.

Advantages over Total B12 testing

	Total B12	Active-B12
Sample denaturation	YES	NO
Analyte	Active and inert B12	Biologically-active portion only
Early marker of B12 status	NO	YES
% indeterminate results ⁹	45%	14%

Performance of Active-B12

Active-B12 offers a more accurate assessment of Vitamin B12 status than Total B12 or MMA⁹.



Using Active-B12

Active-B12 may be used as a direct replacement for the total B12 test or as a secondary test to resolve total B12 indeterminate results¹⁰:

Subjects at risk of B12 deficiency

Active-B12 <35pmol/L

Active-B12 ≥35pmol/L

Likely deficient

Unlikely deficient*

* Renal patients should be further investigated

Total B12 test

<150pmol/L

150 – 300 pmol/L

>300pmol/L

Likely deficient

Resolve with Active-B12

Unlikely deficient*

* Due to false positives and false negatives, all total B12 results could be confirmed with Active-B12

Active-B12 EIA

A novel monoclonal antibody has been developed which is highly specific for holoTC, allowing simple, direct immunoassays for quantifying Active-B12. Such assays remove the need for the cumbersome pre-analytical denaturation steps needed for most Vitamin B12 assays thereby removing a source of variability.



Part Number FMABT100

Method	Enzyme Immunoassay (EIA)
Kit Components	Break-apart wells coated with specific antibody Ready-to-use EIA reagents Compatible with liquid-handling automates
Sample Type	Serum (including gel tubes)
Sample Preparation	1:1 Dilution (diluent provided)
Sample Volume	100µL for duplicate
Calibrators	6, recombinant holoTC, liquid, ready-to-use
Controls	2, recombinant holoTC, liquid, ready-to-use
Substrate	pNPP at 405nm
Reference Method	Abbott AxSYM Active-B12 n=107, r=0.93, EIA = 1.2 + 0.94 x AxSYM

References

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