# Active B12 assay for diagnosing vitamin B12 deficiency

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## Summary

The Abbott ARCHITECT Active-B12 assay is a test for detecting levels of serum holotranscobalamin, which is the metabolically available component of vitamin B12, and can be used as a marker of vitamin B12 deficiency. Four diagnostic test accuracy studies, using different reference standards, reported greater diagnostic accuracy for the Active-B12 assay compared with assays measuring other markers of vitamin B12 deficiency. The assay needs less sample preparation than a total vitamin B12 test and the expected cost is about £3.50 per test including VAT, depending on sample throughput.

| Product summary and likely place in therapy | Accuracy and effectiveness |
|---|---|
| • The Active-B12 assay detects levels of serum holotranscobalamin (holoTC). HoloTC is the metabolically available component of vitamin B12 (cobalamin), and can be used as a marker of vitamin B12 deficiency.<br><br>• The Active-B12 assay would be used in place of current tests in people with suspected vitamin B12 deficiency. | • Eight studies investigated the diagnostic performance of the Active-B12 assay against other blood markers of vitamin B12.<br><br>• Four of the 8 studies that assessed the diagnostic accuracy of the Active-B12 assay against different reference standards reported better accuracy than assays for either cobalamin, folate, homocysteine (Hcy) or methylmalonic acid (MMA).<br><br>• One study found low concordance between Active-B12 holoTC results and MMA levels in samples (n=106) with low or borderline vitamin B12 levels. Active-B12 test results were in the normal range in 13 samples with low total vitamin B12 and raised MMA or Hcy levels, showing the potential for the Active-B12 assay to give false negative results.<br><br>• One study found that MMA was elevated in 31% of samples with an indeterminate holoTC result.<br><br>• Two studies assessed concordance between holoTC and total vitamin B12 levels in different clinical groups and concluded that holoTC was a more sensitive marker of deficiency than total vitamin B12.<br><br>• The evidence suggests that testing for holoTC using the Active-B12 assay may improve diagnostic accuracy when assessing for vitamin B12 deficiency. |

| Technical and patient factors | Cost and resource use |
|---|---|
| • The Active-B12 assay is used in hospital laboratories to test blood samples from patients who are suspected of having vitamin B12 deficiency and who present in community or hospital settings.<br><br>• The target population includes people with suspected pernicious anaemia, with known gastrointestinal and pancreatic disorders associated with vitamin B12 deficiency, or presenting with clinical symptoms of vitamin B12 deficiency.<br><br>• The Abbott ARCHITECT Active-B12 assay can be run on the Abbott ARCHITECT i2000SR or i1000SR analysis systems. The assay needs less sample preparation than a total vitamin B12 test. | • The manufacturer of the Abbott ARCHITECT Active-B12 assay states that an expected cost per test would be around £3.50 including VAT, depending on sample throughput. Running the Active-B12 test needs an Abbott ARCHITECT i2000SR or i1000SR analysis system, the cost of which depends on if it is bought or rented, on reagent purchases and contract length.<br><br>• One of the comparator tests, the Biohit Active-B12, would cost £14.45 per test (excluding VAT). |

## Introduction

Vitamin B12 deficiency can occur if the body does not absorb enough vitamin B12 from the gastrointestinal tract or when there is not enough dietary intake of the vitamin, which is more common in people who have vegan diets and to a lesser extent, vegetarian diets (Devalia et al. 2014; Herrmann et al. 2003).

One cause of vitamin B12 deficiency in the UK is pernicious anaemia. Pernicious anaemia is an autoimmune disorder that results in inflammation and damage to the stomach lining, and loss of cells that produce stomach acid (parietal cells), digestive enzymes and mucus. The parietal cells also

produce intrinsic factor, a protein needed for absorption of vitamin B12 in the gut. Destruction of parietal cells leads to a lack of intrinsic factor.

The exact cause of pernicious anaemia is unknown but, according to the NICE clinical knowledge summary on anaemia, about 30% of people with it have a family history of pernicious anaemia. The condition is more common in people over 60 years, and in women and in people with other autoimmune conditions, such as primary myxoedema, thyrotoxicosis, Hashimoto's disease, Addison's disease and vitiligo. People with pernicious anaemia have a higher risk of developing gastric cancer.

Malabsorption of vitamin B12 may occur in people with gastric, pancreatic or intestinal diseases (including removal of all or part of the stomach or gastric bypass surgery) and in people with HIV. Deficiency can also result from the long-term use of drugs that affect gastric acid production (Kinn and Lantz 1984; Sneiders-Keilholz et al. 1993) or radiotherapy of the abdomen or pelvis, which reduces vitamin B12 absorption from the diet.

The clinical consequences of vitamin B12 deficiency include:

- anaemia resulting from impaired red blood cell production

- loss of peripheral nerve function that can result in impaired sensation, movement or organ function

- visual disturbance

- memory loss

- psychiatric abnormalities.

Vitamin B12 deficiency can also lead to temporary infertility in women. Deficiency during pregnancy can result in foetal abnormalities, such as neural tube defects, according to the NICE clinical knowledge summary on anaemia.

There is uncertainty about the prevalence and incidence of vitamin B12 deficiency. This is partly because there is no established single measure of vitamin B12 deficiency or accepted definition of what constitutes a deficiency (Carmel 2011). Prevalence in the UK is estimated to be around 6% in people under 60 years and closer to 20% in people aged over 60 (Hunt et al. 2014).

There are several approaches to diagnosing vitamin B12 deficiency. These include:

- Tests to detect physiological correlates of vitamin B12 deficiency, for example blood film examination and elevated mean cell volume (MCV). Hypersegemented neutrophils, oval macrocytes and circulating megaloblasts are typical features of vitamin B12 deficiency. However, MCV is not specific to vitamin B12 deficiency and excess alcohol consumption, drug use and myelodysplastic syndrome may also cause increased levels. In addition, increased MCV is often a late indicator of vitamin B12 deficiency (Herrmann and Geisel 2002); 25% of people with vitamin B12-related neurological impairment have normal MCV levels (Devalia et al. 2014).

- Functional tests for biochemical abnormalities associated with vitamin B12 deficiency, for example methylmalonic acid (MMA) or total homocysteine (Hcy) levels:

  - MMA is a substance produced when amino acids are metabolised. It is involved in a reaction that uses vitamin B12 (cobalamin) as a cofactor and so can be used as an indicator of vitamin B12 levels. High levels of plasma MMA may indicate cobalamin deficiency. However, levels may not accurately indicate a deficiency in people aged over 65 years with kidney disease, small bowel bacterial overgrowth or reduced fluid content of the blood because these conditions can also cause elevated MMA levels (Devalia et al. 2014; Hunt et al. 2014).

  - Total serum Hcy is an indicator of vitamin B12 deficiency because cobalamin is needed for the synthesis of methionine from Hcy, and low levels of vitamin B12 lead to increased total serum Hcy. However, its use as a sole confirmatory test is limited because Hcy levels are also higher in people with folate deficiency, vitamin B6 deficiency, renal failure and hypothyroidism (Devalia et al. 2014). Both MMA and Hcy are also late indicators of vitamin B12 deficiency (Herrmann and Geisel 2002).

- Direct tests of vitamin B12 status in its various forms are available (Hunt et al. 2014):

  - Serum total cobalamin or vitamin B12, the test most commonly used in the UK to measure vitamin B12 status, is known as the serum cobalamin level.

  - Serum holotranscobalamin (holoTC): in serum, vitamin B12 exists in 2 bound forms. It can be bound to haptocorrin to form holohaptocorrin; or bound to transcobalamin to form holoTC. Cells can only take up vitamin B12 in the form of holoTC (Hunt et al. 2014). Therefore, measuring holoTC is more reflective of vitamin B12 status than measuring total vitamin B12 or holohaptocorrin alone. Emerging evidence suggests that a low level of holoTC may be a more reliable marker of vitamin B12 deficiency than a low serum cobalamin level, particularly as an early marker (Hunt et al. 2014).

Testing of holoTC is being used more widely although there is a lack of consensus about the cut-off values used to show vitamin B12 deficiency (Hunt et al. 2014), reflecting similar issues with other measures of vitamin B12 status. If the test result falls in the intermediate (borderline) range, a second test, such as measuring MMA levels, is recommended to confirm deficiency (Hunt et al. 2014). The British Society for Haematology guidelines (Devalia et al. 2014) recommend that reference ranges for holoTC are either based on manufacturers' reference ranges or determined by the individual laboratory using the test.

## Technology overview

This briefing describes the regulated use of the technology for the indication specified, in the setting described, and with any other specific equipment referred to. It is the responsibility of healthcare professionals to check the regulatory status of any intended use of the technology in other indications and settings.

## *About the technology*

### CE marking

The Abbott Diagnostics Active-B12 assay for the ARCHITECT i2000SR or i1000SR system (Abbott Diagnostics) was CE marked in September 2011. The assay was previously CE marked for the Abbott AxSYM system, however this version is no longer available. The ARCHITECT assay is a chemiluminescent microparticle immunoassay (CMIA) using magnetic microparticles, whereas AxSYM assay was a microparticle enzyme immunoassay (MEIA) with latex microparticles. As part of the CE-marking of the ARCHITECT assay, the manufacturer had to ensure that both assays gave equivalent results.

The product is regulated under the in vitro diagnostic medical device (98/79/EC) directive and is classed as a general device.

### Description

The Abbott ARCHITECT Active-B12 immunoassay is manufactured by Abbott Diagnostics. This is a high-throughput format that is fully automated and can be run at 200 tests per hour. The assay is designed to be run on the Abbott ARCHITECT i2000SR or i1000SR analyser (Abbott Diagnostics). The assay kit contains all required reagents and these are loaded into the analyser along with the samples. The process of sample preparation, dilution and analysis is automated. In addition, standard laboratory supplies such as micro-pipettes and plasticware are needed.

The Active-B12 test uses a holotranscobalamin (holoTC)-specific monoclonal antibody to capture holoTC from a serum sample. A second monoclonal antibody conjugate is used in the detection step. The assay uses 6 calibrators with defined pmol/l HoloTC concentrations then reads the sample signal against the calibration curve to calculate the sample concentration. A light signal generated from the detection step is directly proportional to the amount of holoTC in the serum sample. The analyser translates the light signal into a numerical sample concentration, expressed in pmol/l. No user input is needed. The output is stored on the instrument and can be printed out or sent directly to the laboratory information management system. The assay range is up to 128 pmol/l but this can be extended to 256 pmol/l by performing a 1:1 dilution. The diluent is an existing consumable called 'Multi-Assay Diluent', which is used in many other ARCHITECT assays.

## Setting and intended use

The Active-B12 assay is a test for vitamin B12 deficiency. It could be used as a replacement for total vitamin B12 testing (Brady et al. 2008) as a first-line or second-line test, whenever testing for vitamin B12 deficiency is clinically indicated in primary or secondary care.

The analysis would be done in secondary care haematology laboratories. Tests would be run by appropriately trained clinical scientists or medical laboratory assistants in automated laboratories, supervised by medical laboratory scientists or clinical scientists.

## Current NHS options

There is currently no standard test for measuring vitamin B12 deficiency. Recent guidelines for the diagnosis and treatment of cobalamin and folate disorders from the British Society for Haematology (Devalia et al. 2014) state that serum total vitamin B12 remains the first-line test because of its wide availability and low cost, but that it lacks the sensitivity and specificity needed for a robust diagnostic test. Plasma methylmalonic acid testing is used to clarify uncertain results, however this may be falsely elevated in people with renal disease, haemoconcentration or small bowel bacterial overgrowth and the high cost of the test has prevented its widespread use (Devalia et al. 2014). The guidelines state that serum holoTC has the potential to be used as a first-line test and may reduce the number of indeterminate results, particularly in people over 65 years. They further state that holoTC has the added advantage that it can be used in pregnant women and in women taking oral contraceptives, because the holoTC fraction of cobalamin does not seem to have the same physiological reduction over the course of pregnancy as total serum cobalamin. These guidelines state that further studies are needed to evaluate the clinical utility of holoTC in assessing vitamin B12 deficiency in a routine high-output laboratory setting.

The NICE clinical knowledge summary on anaemia anaemia states that a diagnosis of vitamin B12 deficiency is generally made through the analysis of a person's clinical history. Signs and symptoms include:

- a 'lemon tinge' to the skin

- inflammation of the tongue

- oropharyngeal ulcers

- neuropsychiatric and neurological symptoms including peripheral neuropathy.

A full blood count should be taken if blood tests show a low haemoglobin level and high mean cell volume (MCV), and serum vitamin B12 and serum folate levels should be checked. In people with a low haemoglobin level and normal or low MCV, ferritin, vitamin B12 and folate levels should be tested.

If a diagnosis of vitamin B12 deficiency is confirmed, the current UK clinical practice is to offer treatment with intramuscular injections of hydroxycobalamin (Devalia et al. 2014; Hunt et al. 2014). Standard therapy is injections of 1000 micrograms given 3 times a week for 2 weeks for people without neurological impairment and the same dose on alternate days until there is no further improvement for people with neurological symptoms. Oral treatment may be considered in certain situations (Hunt et al. 2014).

NICE is aware of the following tests, which fulfil a similar purpose to the Active-B12 (Abbott) test:

- Axis-Shield Active-B12 enzyme immunoassay assay (EIA)

- Biohit Active B12 enzyme-linked immunosorbent assay (ELISA)

- IBL International Active-B12 (holotranscobalamin) ELISA.

## Costs and use of the technology

According to the manufacturer, the expected cost of the Abbott ARCHITECT Active-B12 is around £3.50 including VAT, although no formal list price was made available. The price depends on the type of test, number of samples and order size.

An Abbott ARCHITECT i2000SR or i1000SR analyser is needed to run the Active-B12 assay. The cost of an analyser varies depending on whether it is bought through capital purchase or reagent

rental, as well as on the length of the contract. ARCHITECT i2000SR and i1000SR analysers are commonly used in NHS laboratories.

## Likely place in therapy

Currently vitamin B12 levels are tested when there is clinical suspicion of vitamin B12 deficiency and when monitoring patients during vitamin B12 supplementation therapy.

The Active-B12 assay could be used as a replacement for the current standard test for serum total vitamin B12 levels. Holotranscobalamin levels may be more reliable than serum cobalamin in determining deficiency during pregnancy, and so the Active-B12 test could be offered to this group when available (Devalia et al. 2014).

## Specialist commentator comments

Specialist commentators commented that holoTC testing may be an appropriate replacement for total serum vitamin B12 testing. They noted that a large proportion of total vitamin B12 is in the inactive form and that this can mask a deficiency in bioavailable holoTC, and that holoTC may be the earliest marker for vitamin B12 deficiency.

Two commentators noted that holoTC testing does not eliminate the need for a second confirmatory test, MMA, if results are indeterminate, in which case additional serum creatinine testing may be needed to interpret the MMA result. The size of this indeterminate 'grey zone' is dependent on the holoTC cut-off point chosen, but results that fall in this dynamic range are of uncertain biological significance and need confirmatory testing. One commentator noted that holoTC could be used as a second test for indeterminate total vitamin B12 levels between 150 pmol/l and 200 pmol/l, which may amount to 20% of results in older populations. This may be valuable if there is a discrepancy between a normal total vitamin B12 result and clinical signs of deficiency. However, a different commentator suggested that the use of the Active-B12 assay to confirm indeterminate total vitamin B12 levels was inappropriate because a further group of indeterminate results will need more tests.

Two commentators stated that the assay was of particular value for detecting deficiency in pregnant women, because the holoTC levels, unlike total vitamin B12 levels, do not fall in the second and third trimesters. A second commentator thought that further experience is needed on interpretation of assays in pregnant women.

Commentators noted that although holoTC testing is being increasingly used, there are still discrepancies in the mode of application and the assignment of cut-off values. One specialist commentator noted that variable 'normal' levels of vitamin B12, holoTC and MMA are to be expected in groups with different diets (Herrmann et al. 2003). This commentator stated that although the studies included in this briefing illustrate a range of cut-off points; in their opinion, the cut-off point should be between 19 pmol/l and 34 pmol/l as suggested by Heil et al. (2012) to get the optimal balance between sensitivity and specificity and reduce the need for additional testing with MMA. One commentator noted that individual laboratories should define normal ranges.

On costs, 1 commentator also stated that the price of an Active-B12 assay is likely to be around £10 compared with their own estimate of £2.68 for total vitamin B12, and that the price depends on order size or sample throughput, which is inversely proportional to reagent prices. As such, they noted that the price may be a barrier to the uptake of this new test by NHS laboratories, which are unable to increase their prices to primary care and may be unable to establish a business case for using a better but more expensive test.

## Equality considerations

NICE is committed to promoting equality and eliminating unlawful discrimination. In producing guidance, NICE aims to comply fully with all legal obligations to:

- promote race and disability equality and equality of opportunity between men and women

- eliminate unlawful discrimination on grounds of race, disability, age, sex, gender reassignment, pregnancy and maternity (including women post-delivery), sexual orientation, and religion or belief (these are protected characteristics under the Equality Act 2010).

Testing for vitamin B12 deficiency is recommended for people of all ages in whom deficiency is clinically suspected. Vitamin B12 deficiency is more common in older people. Testing for holoTC may be particularly advantageous for pregnant women. Age, pregnancy and disability resulting from chronic conditions are protected characteristics under the 2010 Equality Act.

# Evidence review

## Clinical and technical evidence

### Regulatory bodies

A search of the Medicines and Healthcare Products Regulatory Agency website revealed no manufacturer Field Safety Notices or Medical Device Alerts for this device. A search of the US Food and Drug Administration (FDA) database: Manufacturer and User Device Facility Experience (MAUDE) identified 2 adverse event reports. One related to a case of falsely elevated Active-B12 results for 5 patients using the ARCHITECT i2000SR analyser. No impact on patient management was reported. The second adverse event report related to a case of falsely elevated Active-B12 results while using the ARCHITECT Active-B12 assay. No adverse impact on patient management was reported.

### Clinical evidence

Twelve studies were identified, 3 of which were excluded because they only compared the results of Active-B12 with alternative measures of either total vitamin B12 or holotranscobalamin (holoTC) but did not provide information specifically on diagnostic accuracy (Al-Aisari et al. 2010; Brady et al. 2008; Fragasso et al. 2012). One study (Greibe and Nexo 2011) was excluded because it specifically evaluated the use of the Active-B12 test in relation to the CobaSorb test for vitamin B12 absorption, rather than evaluating diagnostic accuracy. The remaining 8 studies were considered relevant to this briefing (Bamonti et al. 2010; Heil et al. 2012; Lee et al. 2009; Obeid and Herrmann 2007; Remacha et al. 2014; Sobczynska-Malefora et al. 2014; Valente et al. 2011; Woo et al. 2010; see Appendix for search strategy and selection criteria). These studies investigated the diagnostic accuracy of the Active-B12 assay against other markers of vitamin B12 deficiency in clinical populations.

The study by Bamonti et al. (2010; tables 1 and 2) aimed to establish a cut-off threshold for holoTC for identifying vitamin B12 deficiency using the Active-B12 assay (AxSYM analyser) and to evaluate the analytical performance of the Active-B12 assay.

The correlation between holoTC and total vitamin B12, folate, creatinine and homocysteine (Hcy) levels was assessed using routine blood specimens from 250 people with serum total vitamin B12 concentrations of less than 221 pmol/l. Cut-off points were derived for holoTC, and sensitivity and specificity calculated compared with total vitamin B12 levels. For defining vitamin B12 deficiency using holoTC, a cut-off threshold of 40 pmol/l was chosen. For total vitamin B12, a cut-off threshold of <139 pmol/l was chosen to define 'low' values. Qualitative agreement between holoTC and total

vitamin B12 was 65.2% (p<0.05). Of the 250 people tested, 84 people were identified as having 'normal' levels of both total vitamin B12 and holoTC, and 79 people as having 'abnormal' (low) levels on both tests. In 33 people, total vitamin B12 values were low and holoTC was normal, and in 54 people the opposite was found.

Using this cut-off threshold and comparing total vitamin B12 levels with a lower reference interval of <139 pmol/l, holoTC showed a sensitivity of 0.74 (95% confidence interval [CI] 0.62 to 0.86) and a specificity of 0.52 (95% CI 0.38 to 0.66). The area under the curve (AUC) in receiver operating curve (ROC) analysis for the Active-B12 assay was 0.75 (95% CI 0.63 to 0.87), which was higher than those for folate, Hcy and creatinine.

Agreement between holoTC and folate was 55.2% (p<0.001) and with Hcy 51.6% (p<0.001). The authors noted that the Active-B12 assay was easy to use because of the simplicity of the pre-analytical phase and the automation of the analyser. The authors concluded that the results confirmed the reliability of the Active-B12 assay and stated that it is adequate for routine use in assessing cobalamin deficiency in populations with reduced total vitamin B12 values.

The study by Heil et al. (2012; tables 3 and 4) aimed to validate the diagnostic accuracy of holoTC using the Active-B12 assay (AxSYM analyser) as a screening test for vitamin B12 deficiency. ROC analysis was done on 360 samples collected from patients for whom vitamin B12 testing was requested.

Samples were measured for serum holoTC (using the Active-B12 assay) and total vitamin B12 levels. MMA levels were used as a reference standard to define metabolic vitamin B12 deficiency, with 3 pre-defined cut-off levels to define vitamin B12 deficiency: >0.32 μmol/l (90th percentile), >0.45 μmol/l (97.5th percentile), and >0.77 μmol/l (99th percentile). ROC decision plots were generated for each cut-off.

Using the ROC curve to evaluate different cut-off values for screening (using an MMA threshold of 0.45 μmol/l as the reference standard) the authors agreed that the cut-off offering highest sensitivity with acceptable specificity (>50%) should lie between 19 pmol/l to 36 pmol/l. The authors concluded that holoTC showed better test performance than vitamin B12 and could replace vitamin B12 testing in detecting vitamin B12 deficiency.

The study by Lee et al. (2009; tables 5 and 6) aimed to compare the diagnostic performance of the Active-B12 for holoTC (AxSYM analyser) with total vitamin B12 levels in patients after gastrectomy. Hcy and mean cell volume (MCV) were also measured. They studied 128 patients who

had a gastrectomy after a diagnosis of gastric cancer. Reference values were obtained from 100 healthy people.

HoloTC was measured using the Active-B12 assay (AxSYM analyser, Abbott Diagnostics), total vitamin B12 was measured using the Abbott ARCHITECT B12 kit (Abbott Diagnostics), and serum Hcy was measured using the AxSYM Hcy kit (AxSYM analyser, Abbott Diagnostics). Cut-off values for abnormal total vitamin B12 were <189 pg/ml with the borderline range of 189–400 pg/ml. Cut-off values for abnormality were >14.05 µmol/l for Hcy and >95 fl for MCV.

Serum holoTC was low in 32 patients (25%), whereas total vitamin B12 was low in 10 patients (7.8%) and borderline in 50 patients (39.1%). HoloTC was low in all patients with low total vitamin B12 and normal in all patients with normal total vitamin B12. In the 50 patients with borderline total vitamin B12, 44% were classified as having a low level of holoTC.

Patients with both low holoTC and low total vitamin B12 had significantly higher Hcy levels than patients with normal values for either total vitamin B12, holoTC or both (p<0.001). The authors concluded that serum holoTC was more sensitive than total serum vitamin B12 for detecting vitamin B12 deficiency and was therefore a more effective marker.

The study by Obeid and Herrmann (2007; tables 7 and 8) aimed to evaluate the usefulness of measuring serum holoTC compared with total vitamin B12 in assessing vitamin B12 status in routine laboratory analysis. The authors assessed serum samples from 1018 patients referred to their laboratory in Germany for total cobalamin testing. Levels of total vitamin B12, holoTC, and MMA were measured, and associations between these markers were analysed. Concentrations of serum holoTC were measured using the Active-B12 assay on an AxSYM analyser. MMA was measured using gas chromatography-mass spectrometry. The test for measuring total B12 was not reported in the paper. MMA ≥300 nmol/l was used as a cut-off value to define metabolic cobalamin deficiency.

In patients with normal renal function (number of patients unclear), ROC curve analysis of the holoTC and total vitamin B12 tests in detecting a serum concentration of MMA ≥300 nmol/l showed that the AUC was larger for holoTC (0.71) than for total vitamin B12 (0.60). This indicated a better diagnostic sensitivity and specificity for the holoTC test compared with the total vitamin B12 test. A sensitivity of 72% could be expected by using a cut-off of 35 pmol/l for holoTC and 243 pmol/l for total vitamin B12. Statistical significance was not reported for this analysis.

The authors concluded that compared with total vitamin B12, the holoTC assay was better in detecting elevated concentrations of MMA in patients with normal renal function and that holoTC levels can be used as a first-line parameter in detecting vitamin B12 deficiency.

The study by Remacha et al. (2014; tables 9 and 10) evaluated holoTC levels measured using the Active-B12 assay and conducted a concordance analysis with MMA and Hcy levels in patients with low or borderline levels of serum cobalamin. Serum vitamin B12 levels and red cell folate (RCF) were evaluated using the Elecsys immunoassay (Roche Diagnostics). Serum MMA levels were assessed using a mass spectrometer. High MMA was considered when levels were greater than 0.4 nmol/l. Forty-five healthy people without anaemia, 106 patients with low levels of serum vitamin B12 (≤200 pmol/l), and 27 patients with folate deficiency (RCF <500 nmol/l and vitamin B12 >200 pmol/l) were included. In addition to the lower level of the reference interval for holoTC, several other cut-off points were tested. HoloTC was compared with Hcy and MMA by concordance analysis.

In 71% of patients with low total vitamin B12, serum holoTC was below the cut-off value of 33.5 pmol/l. Of 31 samples with low cobalamin but normal holoTC, MMA or Hcy levels were high in 13 patients, indicating likely cobalamin deficiency.

Concordance analysis was done for 2 cut-off levels of total vitamin B12: ≤200 pmol/l and 150 pmol/l. At the ≤200 pmol/l cut-off, concordance between holoTC and MMA levels was not statistically significant. Concordance between Hcy and holoTC was 62% (Kappa index 0.245, p=0.006). At the ≤150 pmol/l cut-off, concordance between holoTC and MMA levels was not statistically significant. Concordance between holoTC and Hcy was 74% but was not statistically significant (Kappa index 0.215, p=0.08).

The authors concluded that holoTC levels were low in patients with low total vitamin B12 levels and folate levels, but that concordance of holoTC with MMA and Hcy levels in this group was poor. They suggest that these data do not support holoTC as the earliest marker of cobalamin deficiency.

The study by Sobczynska-Malefora et al. (2014; tables 11 and 12) evaluated a service that had substituted serum vitamin B12 measurement with holoTC supported with MMA when holoTC levels were indeterminate. Over 4000 samples received for the assessment of vitamin B12 status in a large London NHS hospital in a 4 -month period were included. Serum holoTC was measured using the Abbott Active-B12 assay (AxSYM analyser). Serum MMA was measured by liquid chromatography-tandem mass spectrometry.

The study categorised samples as 'indeterminate' for holoTC between 25–50 pmol/l. Samples with holoTC in this range also had MMA analysis if the glomerular filtration rate was ≥60 ml/min/1.73m$^2$ or not available. An MMA concentration >280 nmol/l was considered to be elevated. The frequency of elevated MMA when holoTC was indeterminate was assessed in patients with known and unknown renal function.

Of the 4175 samples, 1019 (24%) were in the indeterminate range. Of these, 802 had MMA analysis, because renal function was normal or unknown. Of these, 244 samples had elevated MMA and 534 samples did not show elevated MMA. For samples with indeterminate holoTC, MMA was elevated in 31%. The authors concluded that MMA levels should be tested when samples fall in the indeterminate range.

The study by Valente et al. (2011; tables 13 and 14) investigated the ability of holoTC (measured using the Active-B12 assay), total Hcy, MMA, serum and RCF and other haematological variables to determine vitamin B12 deficiency in an older population.

Non-fasting blood samples and information on diet, lifestyle and medical history were provided by 700 consecutive outpatients attending an outpatient memory clinic in a geriatric unit in Dublin, Ireland. A separate reference population of 120 healthy volunteers was recruited from employees of the Active-B12 assay manufacturer and medical students at the local hospital. This group was used to determine a reference interval for the red cell cobalamin, holoTC and total serum cobalamin assays.

Total serum cobalamin was assessed using the Ciba-Corning radioassay and RCF concentration was assessed by microbiological assays. MMA was assessed using mass spectrometry. Total Hcy and holoTC (Active-B12) were assessed using the AxSYM analyser. Cut-off values were 20 pmol/l for holoTC, 123 pmol/l for serum cobalamin, 0.36 µmol/l for MMA, 15 µmol/l for Hcy, <6.8 nmol/l for serum folate and <340 nmol/l for RCF. The reference standard for cobalamin deficiency was red cell cobalamin <33 pmol/l.

In the outpatient group the prevalence of low red cell cobalamin was 9.6% and the prevalence of low holoTC was 8.1%. The prevalence of raised MMA and Hcy was 41.7% and 52.2% respectively. In the group characterised as vitamin B12 deficient by the reference standard, holoTC, serum cobalamin, serum folate and RCF were all significantly lower and MMA and total Hcy were significantly higher compared with the non-deficient group. The AUC analysis showed that holoTC was the best indicator of tissue vitamin B12 status (AUC 0.90). Differences in AUC between holoTC and serum cobalamin (0.80), MMA (0.78) and total Hcy (0.75) were statistically significant (p<0.001).

The study by Woo et al. (2010; tables 15 and 16) aimed to test the association between levels of holoTC measured using the Active-B12 assay with serum vitamin B12 levels, the precision of the assay, and its diagnostic value. The study included 45 samples from patients, for whom a vitamin B12 test had been requested. It also included 139 samples from patients with normocytic or macrocytic anaemia, who were admitted to a hospital in South Korea.

Serum vitamin B12 levels were measured, as well as holoTC, folate and Hcy levels. Low holoTC was seen in 7 samples (4%) across the 2 groups (184 samples). Of these, only 1 had low serum vitamin B12, 4 were in the borderline range and 2 in the normal range of vitamin B12. In 2 samples with low holoTC, Hcy was high while folate levels were normal. In 10 samples with low folate levels, holoTC levels were within normal range. The authors concluded that holoTC levels were more sensitive than serum vitamin B12, although the study did not use a formal reference standard.

## Recent and ongoing studies

No ongoing or in-development trials for the Active-B12 assay were identified from searches of clinical trials registers. According to the manufacturer, several evaluations of Active-B12 are ongoing at various NHS trusts. Data will be available and published by the end of 2015.

## Costs and resource consequences

Use of the technology could replace total vitamin B12 as the standard test for vitamin B12 deficiency. It is possible that the increased test accuracy might reduce the number of indeterminate results, which may reduce the need for confirmatory tests such as methylmalonic acid. The use of the Active-B12 assay would not eliminate the need for a second confirmatory test, MMA, if results fall within the indeterminate range (Sobczynska-Malefora et al. 2014).

Vitamin B12 testing is currently recommended when clinical signs suggest possible deficiency (Devalia et al. 2014). Use of holoTC testing instead of vitamin B12 testing could result in earlier diagnosis of vitamin B12 deficiency; this could prevent complications of the deficiency and save costs associated with complications.

According to the manufacturer, the ARCHITECT Active-B12 assay does not need sample pre-treatment so it can be run at 200 tests per hour. The ARCHITECT Total B12 test can only be run at 100 tests per hour. Introducing the Active-B12 assay to replace the Total B12 test may therefore have a positive impact on workload, through increased capacity and reduced total analytical time.

A senior NHS laboratory worker advised that total vitamin B12 tests in the hospital setting typically cost from £10–£15. One specialist commentator reported that the NHS laboratory price charged to general practitioners for the total vitamin B12 test is £2.68. This includes reagent cost, labour and overheads. They also noted that private laboratory prices are usually higher, and the primary care price may be discounted or be part of a block contract. According to the manufacturer, the expected cost of the ARCHITECT Active-B12 is around £3.50 including VAT, depending on the number of samples and order size. The manufacturer states that there are about 215 ARCHITECT i2000SR or i1000SR analysers in use in the UK, with around 50% of major hospital laboratories having access to 1 of these analysers.

Pricing arrangements for the Active-B12 test appear to vary depending on whether the test is ordered in a primary or secondary care setting, the laboratory providers, primary care contractual arrangements, and discounts. For this reason and in the absence of formal research into the economic consequences of using the assay, the cost implications of adopting the Active-B12 assay to replace total vitamin B12 testing are unclear.

## Strengths and limitations of the evidence

From the perspective of evaluation of the Active-B12 assay as a diagnostic test, although holoTC is considered to measure bioavailable vitamin B12, there is no clear reference standard and relevant studies have included a variety of comparator tests. Reference intervals and cut-off thresholds for establishing 'low' holoTC varied across studies as did thresholds for other markers of vitamin B12.

Four studies compared the diagnostic performance of holoTC levels, measured with the Active-B12 assay with other markers of vitamin B12 deficiency (Bamonti et al. 2010; Heil et al. 2012; Valente et al. 2011; Obeid and Herrmann, 2007). In the 4 studies, holoTC consistently showed better diagnostic performance. Of these, 1 study suggested that the test showed better performance than folate, Hcy and creatinine (Bamonti et al. 2010), none of which are first-line tests of vitamin B12 deficiency. In the remaining 3 studies, the holoTC assay showed superior accuracy to serum vitamin B12 measures, against different reference standards for vitamin B12 deficiency. One study (Valente et al. 2011) showed that holoTC measurement was better than MMA level testing in an older population.

The study by Remacha et al. (2014) suggests that in a selection of samples with low or borderline total cobalamin 29% might return normal holoTC readings, but only half have normal MMA and Hcy. This raises the possibility that using holoTC may lead to false negatives although firm conclusions are limited by the lack of an accepted reference standard, definition of deficiency and the potential for false positives with MMA and Hcy testing (Devalia et al. 2014).

Several studies were small, resulting in very low numbers of positive test results, and some recruited specific clinical subgroups such as patients who had gastrectomies (Lee et al. 2009) and older people (Valente et al. 2011). There was little detail in all of the studies about the sampling frame, available population or the people recruited, which limits the generalisability of the findings.

No diagnostic intervention studies were found that might give information on the effect of the Active-B12 assay on clinical decision-making or outcomes. No studies had data on assay throughput, time to test results or laboratory factors including workforce requirements.

Of the included studies, 2 declared support from the manufacturer of the Active-B12 assay (Bamonti et al. 2010; Valente et al. 2011), of which 1 study (Valente et al. 2011) included authors who were employed by or consultants to the manufacturer. Two studies (Heil et al. 2012; Obeid and Herrmann, 2007) declared that assays used in the studies were provided by the manufacturer.

## Relevance to NICE guidance programmes

The use of Active-B12 is not currently planned into any NICE guidance programme.

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## Search strategy and evidence selection

### Search strategy

1. The following databases were searched using the keyword 'Active-B12': Ovid Embase (between 1974 and 2015), Ovid MEDLINE(R) (between 1946 to April Week 3 2015), Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations (up to April 22, 2015), CAB Abstracts (up to April 22 2015); Web of Science Core Collection (up to April 22 2015), and Scopus (up to April 22 2015).

2. Reference lists of identified studies were checked to identify any further studies.

3. Information provided by the manufacturer for supporting this briefing report was also checked to identify any further studies.

4. The internet was searched using the above keywords.

5. ClinicalTrials.gov, WHO ICTRP, and Current Controlled Trials were also searched for ongoing trials.

6. The manufacturer's website was thoroughly investigated.

### Evidence selection

The inclusion criteria were as follows:

**Patients:** people for whom tests for vitamin B12 deficiency are needed.

**Intervention:** Active-B12 assay being used on Abbott ARCHITECT i2000SR or i1000SR analyser for testing holotranscobalamin concentration in serum and plasma.

**Comparator:** any other assay for testing holoTC or tests for vitamin B12 deficiency level in blood.

**Outcomes:** any clinical, safety and resource use outcomes, including the following:

- diagnostic test characteristics

- the association between holoTC level and other measures of vitamin B12 status

- assay throughput

- time to test results

- laboratory factors including workforce.

**Study design:** clinical studies including controlled and observational studies were included. Systematic reviews and meta-analyses were used for identifying relevant primary studies only. Proof of concept and non-English language studies were excluded.

## Appendix

## *Contents*

**Data tables**

## Table 1 Summary of the Bamonti et al. (2010) study

| Study component | Description |
|---|---|
| Objectives/ hypotheses | To establish a cut-off threshold for holoTC for identifying vitamin B12 deficiency using the Active-B12 assay (AxSYM analyser) and to evaluate the analytical performance of the Active-B12 assay. |
| Study design | Cross-sectional. |
| Setting | Italy (precise clinical setting unclear). |
| Inclusion/ exclusion criteria | Routine blood specimens with serum total vitamin B12 concentration ≤221 pmol/l. |
| Primary outcomes | Cut-off point for holoTC. Sensitivity, specificity and area under the curve for this cut-off threshold against a reference standard of total vitamin B12. |

| Statistical methods | Analytical imprecision. AUC, sensitivity and specificity of assay using derived cut-off point of ≤40 pmol/l. |
|---|---|
| Conclusions | Results confirmed the reliability of the Active-B12 assay and that it is adequate for routine use in assessing vitamin B12 deficiency in populations with reduced total vitamin B12 values. |
| Abbreviations: AUC, area under the curve; holoTC, holotranscobalamin. | |

## Table 2 Summary of results from the Bamonti et al. (2010) study

| Patients included | • 250 'routine blood specimens'<br><br>• 107 men (mean age 59 years, SD 18.8)<br><br>• 143 women (mean age 54.2 years, SD 23.1).<br><br>No further details reported about sampling or sample characteristics. |
|---|---|
| Primary outcome results | Mean recovery for spiked specimens 95% (95% CI 90–100%).<br>Detection limit 0.07 pmol/l.<br>Cut-off threshold for holoTC ≤0.40 pmol/l.<br>Compared against a total vitamin B12 cut-off threshold of <139 pmol/l.<br>Sensitivity 0.74 (95%CI 0.62–0.86).<br>Specificity 0.52 (95%CI 0.38–0.66).<br>AUC 0.75 (0.63–0.87).<br>AUC for other markers of vitamin B12 deficiency:<br>• folate 0.61 (95%CI 0.47–0.75)<br><br>• homocysteine 0.32 (95%CI 0.19–0.45)<br><br>• creatinine 0.42 (95%CI 0.28–0.56). |
| Abbreviations:AUC, area under the curve; CI, confidence interval; holoTC, holotranscobalamin; SD, standard deviation. | |

## Table 3 Summary of the Heil et al. (2012) study

| Study component | Description |
|---|---|
| Objectives/ hypotheses | To validate the diagnostic accuracy of holoTC as a screening test for metabolic vitamin B12 deficiency. The Abbott AxSYM assay was used for testing holoTC. |
| Study design | Cross-sectional. |
| Setting | Multi-centre, 5 clinical laboratories in the Netherlands. |
| Inclusion/ exclusion criteria | Samples from patients aged ≥18 years, with normal renal function, when vitamin B12 testing has been clinically requested. |
| Primary outcomes | AUC for holoTC and vitamin B12 levels. Reference standard for vitamin B12 deficiency was elevated MMA levels at 3 thresholds. |
| Statistical methods | ROC curve analysis |
| Conclusions | HoloTC has better test performance than vitamin B12 and can replace vitamin B12 testing in detecting vitamin B12 deficiency. Suggested cut-off value 32 pmol/l. |
| Abbreviations: AUC, area under the curve; holoTC, holotranscobalamin; MMA, methylmalonic acid; ROC, receiver operating characteristics. ||

## Table 4 Summary of results from the Heil et al. (2012) study

| Patients included | n=360 Mean age (range) 59 (19–100.5) years. Prevalence of elevated MMA (>0.45 $\mu$mol/l) 13%. |
|---|---|

| Primary outcome results | AUC results: |
|---|---|
| | MMA threshold of >0.32 μmol/l |
| | • holoTC 0.70 (95%CI 0.64–0.87) |
| | • vitamin B12 0.63 (95%CI 0.56–0.70) |
| | • p=0.01. |
| | MMA threshold of >0.45 μmol/l |
| | • holoTC 0.78 (95%CI 0.69–0.87) |
| | • vitamin B12 0.70 (95%CI 0.61–0.79) |
| | • p=0.06. |
| | MMA threshold of >0.77 μmol/l |
| | • holoTC 0.92 (95%CI 0.85–0.98) |
| | • vitamin B12 0.73 (95%CI 0.60–0.87) |
| | • p=0.01. |

Abbreviations:AUC, area under the curve; holoTC, holotranscobalamin; MMA, methlymalonic acid; n, number of patients.

## Table 5 Summary of the Lee et al. (2009) study

| Study component | Description |
|---|---|
| Objectives/ hypotheses | To compare the diagnostic performance of the Active-B12 assay (AxSYM analyser) for holoTC with total vitamin B12 levels in patients after gastrectomy. |
| Study design | Cross-sectional |
| Setting | Department of laboratory medicine, South Korea. |
| Inclusion/ exclusion criteria | Included patients who have had a gastrectomy. Excluded people with estimated glomerular filtration rate <30 ml/min/1.73 m$^2$. |

| Primary outcomes | Serum holoTC |
| --- | --- |
| | Serum total vitamin B12 |
| | Hcy |
| | MCV |
| Statistical methods | Total precision |
| | Lower limit of reference range for holoTC |
| | % agreement of classifications |
| | Correlation between outcomes |
| Conclusions | The authors concluded that serum holoTC is more sensitive than total serum vitamin B12 for detecting vitamin B12 deficiency and is therefore a more effective marker. |
| Abbreviations: holoTC, holotranscobalamin; Hcy, homocysteine; MCV, mean cell volume. | |

## Table 6 Summary of results from the Lee et al. (2009) study

| Patients included | 128 patients after gastrectomy (55 female, 73 male), mean age 60.3 (range 29–86) years, mean time since gastrectomy 30.3 (range 1–100) months. |
| --- | --- |
| | 100 healthy people as controls (58 male, 42 female), age range 24–74 years. |

| | |
|---|---|
| Primary outcome results | Coefficients of variation for total precision of Active-B12 assay:<br><br>• 9.4% for low-level (mean 23.05 pmol/l) controls<br><br>• 7.9% for high-level (mean 54.56 pmol/l) controls.<br><br>From healthy reference group:<br><br>Lower limit of normal 42.48 pmol/l (derived from the lower 95% confidence interval).<br><br>In the sample of patients:<br><br>Serum holoTC: low in 32 (25%) patients.<br><br>Total vitamin B12: low in 10 (7.8%) patients, borderline in 50 (39.1%) patients.<br><br>Serum Hcy: high in 40 (30.4%) patients.<br><br>MCV: high in 35 (27.3%) patients.<br><br>HoloTC was low in all patients with low total vitamin B12 and normal in all patients with normal total vitamin B12.<br><br>In the 50 patients with borderline total vitamin B12, 44% were classified as low for holoTC.<br><br>Of the 22 patients classified as having low holoTC and borderline total B12, 9 were classified as having high Hcy and 7 were clinically suspected of having vitamin B12 deficiency (clinical criteria were not reported).<br><br>Patients with both low holoTC and total vitamin B12 had significantly higher Hcy levels than those with normal values for either total vitamin B12, holoTC or both($p<0.001$).<br><br>Correlations:<br><br>Total vitamin B12 correlation with holoTC: $r=0.6591$; $p<0.001$.<br><br>HoloTC correlation with Hcy: $r=0.4407$; $p<0.001$.<br><br>Total vitamin B12 with Hcy: $r=3599$; $p<0.001$.<br><br>Neither holoTC nor total vitamin B12 was correlated with MCV. |
| Abbreviations:holoTC, holotranscobalamin; Hcy, homocysteine; MCV, mean cell volume. | |

## Table 7 Summary of the Obeid and Herrmann (2007) study

| Study component | Description |
|---|---|
| | |

| | |
|---|---|
| Objectives/ hypotheses | To test the utility of holoTC in assessing vitamin B12 status. |
| Study design | Case series.<br><br>The authors were not aware of the patients' underlying diseases, medications or vitamin supplements.<br><br>Assays:<br><br>• holoTC – a microparticle enzyme immunoassay assay (AxSYM, Abbott)<br><br>• MMA – gas chromatography-mass spectrometry<br><br>• cobalamin – a chemiluminescence immunoassay (ADVIA Centaur System, Bayer). |
| Setting | 1018 serum samples that were referred to the authors' laboratory in a university hospital, Germany for total cobalamin testing, between January and August 2006. |
| Inclusion/ exclusion criteria | Not specified. The study population consisted of non-selected patients. |
| Primary outcomes | Levels of total cobalamin, MMA, and holoTC; correlation between these markers. |
| Statistical methods | ANOVA and post-hoc Tamhane tests were applied. Log transformations were applied on each variable before ANOVA test. Spearman test was used to detect correlations between different markers. |
| Conclusions | Compared to total cobalamin, a better performance of the holoTC assay was seen in detecting elevated concentrations of MMA in patients with normal renal function. Most patients with combined low holoTC and elevated MMA had normal concentrations of total cobalamin. HoloTC can be used as a first-line parameter in detecting cobalamin deficiency. |
| Abbreviations: ANOVA, analysis of variance; holoTC, holotranscobalamin; MMA, methylmalonic acid. | |

## Table 8 Summary of results from the Obeid and Herrmann (2007) study

| Patients included | n=1018 samples from 1018 patients (500 females and 518 males).<br><br>Characteristics ( median [5th–95th percentiles]):<br><br>• age: 58 (24–83) years<br><br>• creatinine: 79.6 (4.2–795.6) µmol/l<br><br>• total cobalamin: 296 (147–858) pmol/l<br><br>• MMA: 249 (111–1290) nmol/l<br><br>• holoTC: 49 (14–142) pmol/l. |
|---|---|
| Primary outcome results | Patients with higher serum creatinine levels had higher levels of MMA and holoTC compared with those who had lower serum creatinine. There was no difference in total cobalamin according to creatinine levels.<br><br>In patients (number unclear) with normal creatinine levels, those identified with MMA >291 nmol/l (the 4th quartile) showed statistically significant differences in holoTC and cobalamin compared with patients with MMA <291 nmol/l.<br><br>The association between serum holoTC and serum MMA was not significant in the total group (r=-0.051; p=0.103). However, this was attributed to renal function, because only patients with increased serum creatinine (number unclear) showed no correlation between serum holoTC and MMA (r=-0.36; p<0.001).<br><br>In patients with MMA ≥300 nmol/l and holoTC ≤35 pmol/l (n=448), total cobalamin was within normal range (median 212; 25th/75th percentiles 171/272 pmol/l).<br><br>In patients (number unclear) with normal renal function, most patients with cobalamin deficiency (holoTC ≤35 pmol/l and MMA ≥300 nmol/l) had cobalamin between 165 and 400 pmol/l. Few patients (n=4) had low cobalamin and normal MMA and holoTC (false positive).<br><br>ROC analysis in detecting a MMA level of ≥300 nmol/l in patients (number unclear) with normal renal function: the AUC was 71% for holoTC and 60% for vitamin B12, indicating a better diagnostic sensitivity and specificity for holoTC compared with total cobalamin (confidence intervals and statistical significance test were not reported). A sensitivity of 72% could be expected by using a cut-off of 35 pmol/l for holoTC and 243 pmol/l for total cobalamin. |

| | |
|---|---|
| Abbreviations:holoTC, holotranscobalamin; MMA, methylmalonic acid; ROC, receiver operating characteristic. | |

## Table 9 Summary of the Remacha et al. (2014) study

| Study component | Description |
|---|---|
| Objectives/ hypotheses | To evaluate holoTC levels measured using the Abbott AxSYM Active-B12 assay and conduct a concordance analysis with MMA and Hcy levels in patients with low or borderline levels of serum cobalamin. |
| Study design | Cross-sectional |
| Setting | Unclear. Haematology department, Barcelona. |
| Inclusion/ exclusion criteria | Reference group: healthy individuals without anaemia. Low levels of vitamin B12 group: cobalamin ≤200 pmol/l. Folate deficiency: RCF <500 nmol/l and cobalamin >200 pmol/l. |
| Primary outcomes | Mean (SD) holoTC levels by group. Kappa index between low holoTC and elevated MMA/ Hcy. |
| Statistical methods | Kappa index |
| Conclusions | HoloTC levels are decreased in patients with low vitamin B12 and folate levels. Concordance of holoTC with MMA and Hcy levels in this group is poor. Around half of samples (n=13) with normal holoTC had elevated MMA or Hcy. This does not support holoTC as the earliest marker of vitamin B12 deficiency. |
| Abbreviations: holoTC, holotranscobalamin; Hcy, homocysteine; MMA, methylmalonic acid; RCF, red cell folate; SD, standard deviation. | |

## Table 10 Summary of results from the Remacha et al. (2014) study

| Patients included | • 45 healthy individuals without anaemia. |
|---|---|
| | • 106 patients with low levels of serum vitamin B12 (≤200 pmol/l. In this group clinical characteristics were recorded to determine their relationship with holoTC levels). |
| | • 27 patients with folate deficiency (RCF <500 nmol/l and vitamin B12 >200 pmol/L). |
| | No further details reported on the people in the study. |
| Primary outcome results | In patients with low vitamin B12, the mean of serum holoTC was 25.1 pmol/l (range 0.18–109.4). |
| | In 75 (71%) of this group serum, holoTC was below the cut-off value of 33.5 pmol/l. Of 31 samples with low cobalamin but normal holoTC, MMA or Hcy levels were elevated in 13, indicating likely cobalamin deficiency. |
| | Mean difference of holoTC compared to healthy group: |
| | • Low vitamin B12 group: 52.6 pmol/l (95%CI 34.3–70.9, p<0.001). |
| | • Folate deficiency group: 39.6 pmol/l (range 10.8–75.5). |
| | Concordance analysis: |
| | Low vitamin B12 cut-off of ≤200 pmol/l: |
| | • MMA with holoTC: concordance 55.6% (Kappa index non-significant). |
| | • Hcy with holoTC: concordance 62% (Kappa index 0.245, p=0.006). |
| | Low vitamin B12 cut-off of ≤150pmol/l: |
| | • MMA levels with holoTC: concordance not reported (Kappa index 0.104, non-significant). |
| | • Hcy with holoTC: concordance 74% (Kappa index 0.215, p=0.08). |
| Abbreviations:holoTC, holotranscobalamin; Hcy, homocysteine; MMA methlymalonic acid; RCF, red cell folate. | |

## Table 11 Summary of the Sobczynska-Malefora et al. (2014) study

| Study component | Description |
|---|---|
| Objectives/ hypotheses | To evaluate the pattern of MMA results from samples with holoTC in the indeterminate range, as measured using the Abbott Active-B12 assay (AxSYM analyser). |
| Study design | Cross-sectional |
| Setting | London NHS hospital laboratory. |
| Inclusion/ exclusion criteria | All samples referred for vitamin B12 status evaluation in a 4-month period. |
| Primary outcomes | HoloTC (Active-B12)<br>MMA |
| Statistical methods | Spearman's correlations |
| Conclusions | Indeterminate holoTC (25–50 pmol/l) is a poor predictor of MMA levels according to the thresholds for elevation used in this study. |
| Abbreviations: holoTC, holotranscobalamin; MMA, methlymalonic acid. | |

## Table 12 Summary of results from the Sobczynska-Malefora et al. (2014) study

| Patients included | 4175 samples routinely referred for analysis of vitamin B12 status; median age 56 (range 0–101) years; 55% female. |
|---|---|

| Primary outcome results | 205(5%) samples had low holoTC (<25 pmol/l). |
|---|---|
| | 1019 (24%) were in the indeterminate range. |
| | 802 had MMA analysis because renal function was normal or unknown. |
| | 244 samples had elevated MMA and 534 did not show elevated MMA. |
| | Incidence of elevated MMA according to holoTC level: |
| | holoTC 25–29 pmol/l: 41% |
| | holoTC 30–34 pmol/l: 32% |
| | holoTC 35–39 pmol/l: 33% |
| | holoTC 40–44 pmol/l: 30% |
| | holoTC 45–50 pmol/l: 26% |
| | holoTC was not correlated with MMA levels. |
| Abbreviations:holoTC, holotranscobalamin; Hcy, homocysteine; MMA methlymalonic acid. | |

## Table 13 Summary of the Valente et al. (2011) study

| Study component | Description |
|---|---|
| Objectives/ hypotheses | To investigated the ability of holoTC (measured using the Abbott AxSYM Active-B12 assay), total Hcy, MMA, serum and erythrocyte folate and other haematological variables to discriminate cobalamin deficiency in an older population. |
| Study design | Cross-sectional |
| Setting | Geriatric outpatient memory clinic. Clinical laboratory. |
| Inclusion/ exclusion criteria | 700 consecutive patients attending a geriatric outpatient memory clinic. |
| | 120 healthy volunteers from manufacturer employees and medical students. |
| Primary outcomes | holoTC (measured using the Active-B12 assay), total Hcy, MMA, serum and erythrocyte folate. |
| | Reference standard: red cell cobalamin <33 pmol/l. |
| Statistical methods | ROC plots; sensitivity, specificity and proportion of results correctly allocated; PPV; NPV. |

| Conclusions | HoloTC performed significantly better than all other indicators in this group of older people. |
|---|---|
| Abbreviations: Hcy, homocysteine; holoTC, holotranscobalamin; MMA, methlymalonic acid; NPV, negative predictive value; PPV, positive predictive value; RCF, red cell folate; ROC, receiver operating characteristic. | |

## Table 14 Summary of results from the Valente et al. (2011) study

| Patients included | Outpatients: n=700; mean age 81 (2.5th to 97.5th percentile 69–92) years; 490 (70%) female. | | |
|---|---|---|---|
| | Reference population: n=120; median age 31 (range 18–62) years, 53% female. | | |
| **Primary outcome results:** | | | |
| HoloTC (pmol/l) (mean and 95% CI) | Vitamin B12 deficient | Vitamin B12 non-deficient | Analysis |
| | 18.2 (15.6–21.2) | 51.4 (49–53.9) | p=0.0001 |
| Prevalence | Prevalence:<br><br>• low holoTC: 8.1%<br><br>• low total cobalamin: 8%<br><br>• low red cell cobalamin: 9.6%<br><br>• elevated MMA: 41.7%<br><br>• elevated Hcy: 52.2%. | | |
| ROC analysis–AUC [a] | • holoTC 0.90<br><br>• serum cobalamin 0.80<br><br>• MMA 0.78<br><br>• total Hcy 0.75. | | |
| ROC analysis– diagnostic performance at a single cut-off [a] | | Threshold 19.6 pmol/l | Threshold 29.9 pmol/l |

| | | | |
|---|---|---|---|
| • Sensitivity (%) | holoTC | 50.7 | 80.6 |
| | Serum cobalamin | 13.4 | 86.6 |
| | MMA | 17.9 | 88.1 |
| • Specificity (%) | holoTC | 96.4 | 84.5 |
| | Serum cobalamin | 99.1 | 57.3 |
| | MMA | 98.7 | 17.9 |
| • PPV (%) | holoTC | 60 | 36 |
| | Serum cobalamin | 60 | 18 |
| | MMA | 60 | 16 |
| • NPV (%) | holoTC | 95 | 98 |
| | Serum cobalamin | 92 | 98 |
| | MMA | 92 | 98 |

Abbreviations: AUC, area under the curve; CI, confidence interval; holoTC, holotranscobalamin; Hcy, homocysteine; MMA methlymalonic acid; PPV, positive predictive value; ROC, receiver operating characteristic; NPV, negative predictive value.

[a] 95% CIs not reported.

## Table 15 Summary of the Woo et al. (2010) study

| Study component | Description |
|---|---|
| Objectives/ hypotheses | To test the association between levels of holoTC measured using the Abbott AxSYM Active-B12 assay with serum vitamin B12 levels, perform a precision test of the assay and determine the diagnostic value of the assay. |
| Study design | Cross-sectional |
| Setting | Clinical laboratory and a South Korean Hospital. |

| Inclusion/ exclusion criteria | Samples from patients, for whom a vitamin B12 test had been requested because of conditions such as Alzheimer's disease, dementia, Parkinson's disease, cancer, unstable angina and infarction. |
|---|---|
| | Samples from patients with normocytic or macrocytic anaemia, who were admitted to a hospital in South Korea between August 2007 and March 2008. |
| Primary outcomes | holoTC, cut-off 35 pmol/l; vitamin B12, cut-off 150 pmol/l; folate, cut-off 3 µg/l; Hcy, cut-off 12 µmol/l. |
| Statistical methods | Assay imprecision<br>Chi-square test for association between holoTC and other markers. |
| Conclusions | HoloTC was more sensitive than serum vitamin B12 (no formal reference standard applied). |
| Abbreviations: holoTC, holotranscobalamin; Hcy, homocysteine. | |

## Table 16 Summary of results from the Woo et al. (2010) study

| Patients included | n=184 (90 female, 94 male). |
|---|---|
| | 45 samples from patients, for whom a serum vitamin B12 test had been requested because of conditions such as dementia with Alzheimer's disease, Parkinson's disease, cancer (including adenocarcinoma of the stomach), unstable angina, and infarction. |
| | 139 samples from patients with normocytic or macrocytic anaemia, who were admitted to Dong-A University Hospital between August 2007 and March 2008. |
| Primary outcome results | Within-run and between-run imprecision values all less than 3.5%. |
| | Low holoTC (<35 pmol/l) was seen in 7 (4%) of samples. Of these, only 1 had both low holoTC and low serum vitamin B12; 4 showed borderline and 2 normal vitamin B12 levels. |
| | In 2 patients with low holoTC, Hcy was elevated (>12.0 nmol/l) indicating vitamin B12 deficiency, while folate levels were normal. |
| | In 10 samples with low folate levels (<3.0 µg/l), holoTC levels were within normal range. |
| | Chi-square test to determine relationship between low holoTC and vitamin B12 levels: $p=0.0001$. |
| Abbreviations:holoTC, holotranscobalamin; Hcy, homocysteine. | |

## About this briefing

Medtech innovation briefings summarise the published evidence and information available for individual medical technologies. The briefings provide information to aid local decision-making by clinicians, managers and procurement professionals.

Medtech innovation briefings aim to present information and critically review the strengths and weaknesses of the relevant evidence, but contain no recommendations and **are not formal NICE guidance.**

## *Development of this briefing*

This briefing was developed for NICE by the Birmingham and Brunel Consortium. The interim process & methods statement sets out the process NICE uses to select topics, and how the briefings are developed, quality-assured and approved for publication.

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