MBS Reviews

VITAMIN B12 TESTING

REPORT

February 2014
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>4</td>
</tr>
<tr>
<td>EXECUTIVE SUMMARY</td>
<td>5</td>
</tr>
<tr>
<td>PURPOSE OF THE REVIEW</td>
<td>5</td>
</tr>
<tr>
<td>VITAMIN B12 TESTING</td>
<td>5</td>
</tr>
<tr>
<td>CONCERNS ABOUT VITAMIN B12 TESTING</td>
<td>5</td>
</tr>
<tr>
<td>REVIEW METHODOLOGY</td>
<td>6</td>
</tr>
<tr>
<td>STAKEHOLDER CONSULTATION</td>
<td>6</td>
</tr>
<tr>
<td>SUMMARY OF FINDINGS</td>
<td>6</td>
</tr>
<tr>
<td>CONCLUSIONS</td>
<td>8</td>
</tr>
<tr>
<td>1 BACKGROUND ON VITAMIN B12 TESTING</td>
<td>10</td>
</tr>
<tr>
<td>1.1 DESCRIPTION OF CURRENT SERVICES</td>
<td>10</td>
</tr>
<tr>
<td>1.2 THE CLINICAL FLOWCHARTS</td>
<td>16</td>
</tr>
<tr>
<td>2 REVIEW METHODOLOGY</td>
<td>18</td>
</tr>
<tr>
<td>2.1 SECONDARY DATA ANALYSIS</td>
<td>18</td>
</tr>
<tr>
<td>2.2 GUIDELINE CONCORDANCE</td>
<td>18</td>
</tr>
<tr>
<td>2.3 SYSTEMATIC LITERATURE REVIEW FOR CLINICAL EVIDENCE</td>
<td>19</td>
</tr>
<tr>
<td>2.4 SYSTEMATIC LITERATURE REVIEW FOR ECONOMIC EVIDENCE</td>
<td>22</td>
</tr>
<tr>
<td>3 SECONDARY DATA ANALYSIS</td>
<td>23</td>
</tr>
<tr>
<td>3.1 MBS ITEM NUMBER USAGE AND EXPENDITURE</td>
<td>23</td>
</tr>
<tr>
<td>3.2 AGE AND GENDER PROFILE OF PATIENTS</td>
<td>26</td>
</tr>
<tr>
<td>3.3 FREQUENCY OF TESTING BY PATIENT</td>
<td>27</td>
</tr>
<tr>
<td>3.4 PROFILE OF PROVIDERS REQUESTING VITAMIN B12/FOLATE TESTING SERVICES</td>
<td>28</td>
</tr>
<tr>
<td>3.5 FREQUENCY OF REQUESTS FOR TESTING BY PROVIDER</td>
<td>29</td>
</tr>
<tr>
<td>4 REVIEW OF GUIDELINES RELEVANT TO VITAMIN B12 TESTING</td>
<td>31</td>
</tr>
<tr>
<td>4.1 CLINICAL PRACTICE GUIDELINES</td>
<td>31</td>
</tr>
<tr>
<td>4.2 RECOMMENDATIONS FROM OTHER REPORTS</td>
<td>35</td>
</tr>
<tr>
<td>4.3 SUMMARY OF CLINICAL GUIDANCE</td>
<td>36</td>
</tr>
<tr>
<td>5 REVIEW OF THE CLINICAL EVIDENCE FOR VITAMIN B12 TESTING</td>
<td>37</td>
</tr>
<tr>
<td>5.1 EVIDENCE BASE</td>
<td>37</td>
</tr>
<tr>
<td>5.2 APPROPRIATE CLINICAL INDICATIONS FOR VITAMIN B12 TESTING</td>
<td>37</td>
</tr>
<tr>
<td>5.3 EVIDENCE THAT TESTING FOR VITAMIN B12 LEVELS IMPROVES HEALTH OUTCOMES</td>
<td>37</td>
</tr>
<tr>
<td>5.4 RISKS/HARMS ASSOCIATED WITH VITAMIN B12 TESTING</td>
<td>37</td>
</tr>
<tr>
<td>5.5 QUALITY OF TESTING ACCORDING TO TESTING PLATFORM</td>
<td>38</td>
</tr>
</tbody>
</table>
6 REVIEW OF THE ECONOMIC EVIDENCE FOR VITAMIN B12 TESTING .................. 43
6.1 STUDIES RELEVANT TO THE ECONOMIC EVALUATION OF VITAMIN B12 TESTING ........................................ 43

7 FINDINGS AND CONCLUSIONS ......................................................................................................................... 44
7.1 CURRENT USAGE OF VITAMIN B12 AND/OR FOLATE TESTING SERVICES IN AUSTRALIA ....................... 44
7.2 CLINICAL GUIDANCE ON VITAMIN B12 TESTING ............................................................................................ 45
7.3 RELATIONSHIP BETWEEN TESTING FOR VITAMIN B12 LEVELS AND HEALTH OUTCOMES ..................... 46
7.4 HARRMS ASSOCIATED WITH VITAMIN B12 TESTING ....................................................................................... 46
7.5 DIAGNOSTIC PERFORMANCE OF VITAMIN B12 TESTS .................................................................................... 46
7.6 COST IMPLICATIONS OF VITAMIN B12 TESTING .............................................................................................. 47
7.7 CONCLUSIONS ....................................................................................................................................................... 47

APPENDIX 1 – REFERENCES ................................................................................................................................... 48

APPENDIX 2 – REVIEW CONSULTATION COMMITTEE MEMBERS .......................................................................... 55

APPENDIX 3 – MBS INFORMATION ........................................................................................................................ 56

APPENDIX 4 – SEARCH TERM STRATEGY ............................................................................................................. 57

APPENDIX 5 – TOOLS FOR ASSESSING THE EVIDENCE IN THE SYSTEMATIC REVIEW ................................ 62
**LIST OF ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANZFSC</td>
<td>Australian and New Zealand Food Standards Code</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the curve</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CMFM</td>
<td>Comprehensive Management Framework for the MBS</td>
</tr>
<tr>
<td>holoTC</td>
<td>Holotranscobalamin</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>HTA</td>
<td>Health technology assessment</td>
</tr>
<tr>
<td>Hyc</td>
<td>Homocysteine</td>
</tr>
<tr>
<td>MBS</td>
<td>Medicare Benefits Schedule</td>
</tr>
<tr>
<td>MMA</td>
<td>Methylmalonic acid</td>
</tr>
<tr>
<td>MSAC</td>
<td>Medical Services Advisory Committee</td>
</tr>
<tr>
<td>NHMRC</td>
<td>National Health and Medical Research Council</td>
</tr>
<tr>
<td>NLR</td>
<td>Negative likelihood ratio</td>
</tr>
<tr>
<td>PA</td>
<td>Pernicious anaemia</td>
</tr>
<tr>
<td>PASC</td>
<td>Protocol Advisory Sub-Committee (of MSAC)</td>
</tr>
<tr>
<td>PICO</td>
<td>Population, Intervention, Comparator, Outcomes</td>
</tr>
<tr>
<td>PLR</td>
<td>Positive likelihood ratio</td>
</tr>
<tr>
<td>QUOROM</td>
<td>Quality of Reporting of Meta-analyses</td>
</tr>
<tr>
<td>RCC</td>
<td>Review Consultation Committee</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomised controlled trials</td>
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</tbody>
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EXECUTIVE SUMMARY

In the 2011-12 Budget, the Australian Government announced a further commitment to the Comprehensive Management Framework for the Medicare Benefits Schedule [MBS] (CMFM), to continue the systematic review of MBS items to ensure that they reflect contemporary evidence, improve health outcomes for patients and represent value for money.

MBS Reviews aim to ensure the clinical and financial sustainability of the MBS. Reviews assess specific MBS services (i.e. MBS items) and associated policy issues in a focussed, fit-for-purpose, evidence-based process. Findings recognise that the MBS funding should align with contemporary evidence, reflecting appropriate patient groups and best clinical practice.

The Reviews have a primary focus on improving health outcomes and the financial sustainability of the MBS, through the following criteria:

- assess patient safety risk;
- identify services that have limited health benefit and/or are used inappropriately;
- be evidence-based and fit-for-purpose;
- be conducted in consultation with key stakeholders including, but not limited to, the medical profession and consumers;
- include opportunities for public submission; and
- use Government resources efficiently.

Purpose of the review

This Review Report outlines the rationale behind conducting the review of the MBS items relevant to vitamin B12 testing services (refer to Appendix 3 for MBS item descriptor) and the process undertaken to identify and appraise the available information on the MBS item to ensure that it reflects contemporary evidence, improves health outcomes for patients, and represents value for money.

Vitamin B12 testing

Vitamin B12, also called cobalamin, is a water soluble vitamin that plays a fundamental role in the normal functioning of the brain and nervous system, and for the formation of blood. Vitamin B12 deficiency can be the result of nutritional deficiency, increased requirements, impaired absorption, or other gastrointestinal causes. Deficiency is associated with a wide spectrum of haematologic, neurologic and psychiatric disorders that can often be reversed by early diagnosis and prompt treatment. The diagnosis of vitamin B12 deficiency has traditionally been based on measuring total serum levels of vitamin B12.

Concerns about vitamin B12 testing

Concerns raised with vitamin B12 testing relate to the increase in the number of claims and benefits paid for MBS item numbers 66599 and 66602. Given the widespread testing of vitamin B12/folate by general practitioners, this review is focused on identifying appropriate clinical indications for medically necessary vitamin B12 testing, and determining whether testing should be limited to certain high risk groups.
Review methodology

The review methodology comprised of consulting with key stakeholders; developing a review protocol, which outlined the detailed review methodology (including specifying the key clinical/research questions for the systematic review, preparing the clinical flowcharts, and documenting the economic analysis strategy); analysing secondary data sources (Medicare Australia); conducting an evidence-based systematic literature review on vitamin B12; and undertaking an assessment and analysis of the evidence to draw conclusions in relation to the clinical/research questions.

Stakeholder consultation

Stakeholder engagement is a pivotal part of the MBS Reviews process, particularly as feedback helps inform Review Reports. During the review process, stakeholders were informed of the progress of the MBS items being considered. This included ensuring that relevant documents were released for public consultation at the appropriate time and that comments were incorporated into the review process.

As part of the MBS Review process, the Department established a Review Consultation Committee (RCC). The RCC is a time-limited committee of nominated representatives, established to provide advice to the Department. A list of RCC members is found at Appendix 2.

Summary of findings

Current usage of vitamin B12 and/or folate testing services in Australia

Over the past 10 years, the number of claims for MBS item 66599 has more than doubled (+119%) from 282,531 in 2003/04 to 618,744 in 2012/13. Over the same timeframe the number of claims for MBS item 66602 has had an even greater increase (+307%) from 522,980 to 2,129,051. The increase in benefits paid for both items reflects the increase in claims (+120% for item 66599 and +309% for item 66602). While total benefits increased significantly, the proportion of benefits paid to each state and territory remained relatively constant over the ten-year period. The highest proportion of benefits paid over the past ten years was in New South Wales (34% and 38% of total benefits for 66599 and 66602, respectively), followed by Victoria (30% for 66599 and 28% for 66602).

An analysis was conducted of the number of services per capita (i.e. per 100,000 population). Across Australia, there were 2,666 claims for item 66599 per 100,000 people enrolled in Medicare in 2012/13 and 9,172 claims per 100,000 for item 66602. South Australia had the highest per capita rate of claiming for item 66599, while the Northern Territory had the lowest. For item 66602, the highest number of claims per capita was for NSW and Victoria, while Tasmania had the lowest.

MBS item numbers 66599 and 66602 are claimed by both males and females; however, females had a higher number of tests at all ages, except in the youngest age category (< 5 years). Females also had a steeper increase in testing volume than males, with the largest difference between genders in the 15-24 and 25-34 year age groups. For item 66599, the number of tests being performed in people aged 45 years and over was 76% for males and 64% for females. For item 66602, 71% of claims for males were aged 45 years and over versus 60% for females.
For both MBS items, there was an increase in the overall number of patients tested between 2008/09 and 2012/13. However, there was very little change in the proportion of patients receiving either one test per year (approximately 91%), two tests per year (approximately 8%), or three or more tests per year (approximately 1%). These data suggest that the majority of vitamin B12/folate testing services are being undertaken for the purposes of screening/testing rather than monitoring.

Over the five-year time period from 2008/09 to 2012/13, there were no material changes in the pattern of requesting providers. General practitioners and other medical practitioners accounted for approximately 71% and 67% of all providers requesting item 66599 and item 66602, respectively. Approximately 14% of providers requesting vitamin B12/folate testing were internal medicine consultant physicians. There was a large variety of other providers requesting services, but they each accounted for less than 4% of provider counts.

For both items, there was an increase over the period 2008/09 to 2012/13 in the overall number of providers requesting vitamin B12/folate testing. The majority of providers (97% and 88% for item 66599 and 66602, respectively) requested 100 or fewer tests per year. There were a small number of providers that requested more than 400 tests per year (601 providers in 2012/13).

**Clinical guidance on vitamin B12 testing**

The MBS data indicates that the majority of requests for vitamin B12 testing are initiated by general practitioners and other medical practitioners. However, no relevant Australian guidelines were identified in the literature search. Four international guidelines on diagnosing vitamin B12 deficiency were identified (two from the UK and two from Canada), with limited evidence supporting the recommendations. One 2012 guideline from the British Columbia Medical Association and Ministry of Health, Canada, concluded that routine screening for vitamin B12 deficiency is not recommended. No other guidelines made recommendations relating to routine screening.

Several guidelines recommend that patients with symptoms or signs of vitamin B12 deficiency anaemia (macrocytic anaemia or macrocytosis) and patients with suspected neuropsychiatric abnormalities should be tested for vitamin B12 deficiency. Other populations where testing could be considered include the elderly, long-term vegans, people on drugs that interfere with vitamin absorption (long-term H2 receptor antagonists or proton pump inhibitors or metformin) and patients with inflammatory bowel disease, gastric or small intestine resection.

The frequency with which patients should be tested was not addressed in the guidelines. However, a 2005 best practice review from the UK stated that there is no obvious merit in repeating vitamin B12 measurements in patients with macrocytic anaemia, macrocytosis, or patients with specific neuropsychiatric abnormalities, unless lack of compliance is suspected or anaemia recurs.

Only one guideline specified a methodology for vitamin B12 measurement. A practice parameter, published by the American Academy of Neurology recommended using serum vitamin B12 level with metabolites (MMA and Hcy) in the evaluation of vitamin B12 deficiency in all patients with distal symmetric neuropathy. None of the guidelines advised on the diagnostic accuracy of the different tests used to assess vitamin B12 deficiency or
advised on which metabolite is the best indicator of vitamin B12 status. This may be attributed to the lack of a gold reference standard for vitamin B12.

In July 2013, Health Quality Ontario released recommendations on a variety of common clinical tests, including vitamin B12. On the basis of their rapid review which found that serum vitamin B12 test has low diagnostic accuracy, it was recommended that vitamin B12 testing is removed from the Ontario laboratory requisition form.

**Relationship between testing for vitamin B12 levels and health outcomes**

No definitive conclusions can be drawn about the effectiveness of vitamin B12 testing since no prospective comparative trials have been conducted to directly assess the impact of testing on health outcomes in healthy populations or in patients with chronic disease associated with vitamin B12 deficiency.

No trials designed to directly measure the risks or harms associated with vitamin B12 testing were identified. However, vitamin B12 testing relies on a blood draw, which is a safe procedure. Vitamin B12 supplements are generally considered safe when taken in amounts that are not higher than the recommended dietary allowance. Thus, it is likely that the consequences of inaccurate or inappropriately interpreted serum vitamin B12 test results, such as a false positive, are relatively small.

It is unknown whether measurement of vitamin B12 levels is cost-effective. No costing studies or economic analyses relating to vitamin B12 testing were identified.

**Diagnostic performance of vitamin B12 tests**

There is currently no consensus on the best method to estimate vitamin B12 deficiency and vitamin B12 status. A 2011 systematic review evaluated the diagnostic accuracy of serum B12 tests using MMA, Hcy and holoTC as reference standards. The review noted that measurement of total serum vitamin B12 is widely used as a standard screening test; however, the evidence base for the diagnostic accuracy of this test is low, due to the lack of a gold reference standard. From the available evidence, diagnosis of conditions amenable to vitamin B12 supplementation on the basis of serum vitamin B12 levels alone cannot be considered a reliable approach to investigating suspected vitamin B12 deficiency. Across clinical indications, practice settings and different methodologies, the authors of the review found low levels of test sensitivity and, to a lesser extent, specificity. The authors of the review also demonstrated that the transition from older assay methods to newer technologies, such as chemiluminescence, was not associated with improved diagnostic accuracy. The lack of a gold standard also hampers the ability to compare the various markers of B12 deficiency (MMA, Hcy, and holoTC) with each other.

There is some evidence that holoTC has comparable or better diagnostic accuracy to that of total serum vitamin B12. However, there is insufficient evidence to establish holoTC testing as an alternative to either total serum vitamin B12, or levels of MMA or homocysteine in the diagnosis of vitamin B12 deficiency.

**Conclusions**

There has been a substantial increase in the number of claims for vitamin B12/folate testing over the past ten years. Analysis of MBS data indicates that the majority of vitamin B12 testing services are requested by GPs and OMPs for the purposes of screening or testing,
rather than follow-up monitoring. There are no Australian clinical practice guidelines that either advocate or recommend against routine testing for vitamin B12. The international clinical practice guidelines vary widely in their recommendations. While some recommend vitamin B12 test as screening tools in commonly encountered illnesses such as dementia, others suggest restricting testing to patients who have already undergone pre-test investigations such as a full blood count or blood film examination. There are no recommendations on the frequency of vitamin B12 testing and there is no direct evidence regarding the clinical utility of vitamin B12 testing in any population.
1 BACKGROUND ON VITAMIN B12 TESTING

1.1 Description of current services

This section describes vitamin B12 and vitamin B12 testing, recommended vitamin B12 status, and the population groups and clinical conditions/risk factors in which vitamin B12 testing is recommended.

1.1.1 The mechanism of vitamin B12 absorption

Vitamin B12, also called cobalamin, is a water soluble vitamin that plays a fundamental role in the normal functioning of the brain and nervous system, and for the formation of blood. Dietary vitamin B12 initially binds a protein called haptocorrin (previously known as transcobalamin I or R-Factor), which is produced by the salivary glands of the oral cavity (as well as the parietal cells of the stomach), and whose essential function is to protect vitamin B12 from degradation from the acidic environment of the stomach. Absorption of vitamin B12 occurs in the terminal ileum (i.e. most distal part of the small intestine) and is aided by the binding of intrinsic factor (IF) secreted by the parietal cells of the stomach to the vitamin.\(^1\) (Figure 1.1). In addition to this method of absorption, evidence supports the existence of an alternate pathway that is independent of the IF. This pathway is important in relation to oral supplementation (approximately 1% of a large oral dose of vitamin B12 is absorbed by this second mechanism).\(^2\) Once absorbed, vitamin B12 binds to a protein called transcobalamin II (holoTC or active B12 is the complex formed by the binding of vitamin B12 to transcobalamin II), and is transported throughout the body. The interruption of one or any combination of these steps places a person at risk of developing vitamin B12 deficiencies.\(^3\)

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\(^1\) Parietal cells are cells in the stomach that produce gastrichydrochloric acid and also produce the intrinsic factor, which rebinds vitamin B12 after it is released from haptocorrin by digestion.
Figure 1.1: Vitamin B12 absorption and transport\(^{(3)}\)

![Diagram of vitamin B12 absorption and transport](image)

1.1.2 The functions of vitamin B12 in the human body

In humans, vitamin B12 and folate are linked by two enzymatic reactions where they function as cofactors (i.e. a cofactor is a component, other than the protein portion, of many enzymes to facilitate the catalytic activity of the enzyme)\(^{(4)}\). Vitamin B12 is required as a cofactor in both reactions, whereas folate is required in only one of the reactions (see Figure 1.2).\(^{(3)}\)

Figure 1.2: The enzymatic reactions that require vitamin B12 and folate (folic acid) as cofactors\(^{(5)}\)

\[
\text{Methylmalonic acid} \rightarrow \text{Methylnalonyl-CoA} \rightarrow \text{Succinyl-CoA} \rightarrow \text{Homocysteine + folic acid} \rightarrow \text{Methionine}
\]

In the first reaction, vitamin B12 is required for the conversion of methylmalonic acid (MMA) to succinyl-CoA. MMA is a substance produced when proteins in the body are broken down.\(^{(6)}\) Folate does not play any role in this reaction. Deficiency in vitamin B12 can lead to increased levels of serum MMA.\(^{(3)}\)
In the second reaction, both vitamin B12 (in the form of methylcobalamin) and folic acid act as cofactors in the conversion of the substrate homocysteine (a homologue of the amino acids cysteine and methionine) to methionine (an amino acid and one of the 20 building blocks of proteins) by the enzyme methionine synthase. More importantly, this pathway is closely linked to the generation of thymidine which is vital for deoxyribonucleic acid (DNA, i.e. the building block of the human body which carries genetic information) synthesis. A deficiency in either vitamin B12 or folic acid or both can lead to increased homocysteine levels in plasma. In addition, deficiency of either vitamins can result in perturbation of these two key pathways with consequent disruption of DNA synthesis caused by thymidine lack and resulting in megaloblastic anaemia, as well as other adverse effects on the nervous system and other organs. It is this metabolic reaction that clearly links the two vitamins and is responsible for the common or shared neuropsychiatric and haematologic disorders discussed in the following sections.

1.1.3 Vitamin B12: dietary sources, fortification, and supplements

Vitamin B12 is present in animal products such as meat, poultry, fish (including shell fish), and to a lesser extent milk, cheese and eggs, and it is not present in plant products. The recommended dietary allowance for vitamin B12 is 2.4 µg/day and most individuals can meet this level through dietary intake. Table 1.1 lists some of the foods with substantial amounts of vitamin B12, along with their vitamin B12 content. Individuals over the age of 50 who have reduced protease secretions in the stomach (as well as strict vegetarians) obtain their vitamin B12 from supplements or fortified foods (e.g. fortified cereal) because of the increased likelihood of food-bound vitamin B12 malabsorption.

Table 1.1: Examples of dietary sources of vitamin B12

<table>
<thead>
<tr>
<th>Type of food</th>
<th>Estimated vitamin B12 content (micrograms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clams, (85 grams)</td>
<td>84.0</td>
</tr>
<tr>
<td>Mussels, (85 grams)</td>
<td>20.4</td>
</tr>
<tr>
<td>Crab, (85 grams)</td>
<td>8.8</td>
</tr>
<tr>
<td>Salmon (85 grams)</td>
<td>2.4</td>
</tr>
<tr>
<td>Beef, (85 grams)</td>
<td>2.1</td>
</tr>
<tr>
<td>Chicken</td>
<td>0.3</td>
</tr>
<tr>
<td>Egg (whole)</td>
<td>0.6</td>
</tr>
<tr>
<td>Milk (85 grams), 1 glass</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Food fortification is defined as the process of adding micronutrients (such as vitamins and minerals) to food as permitted by the Australian and New Zealand Food Standards Code (ANZFSC). Regulations regarding the fortification of foods with vitamin B12 vary between countries. ANZFSC permits only a limited number of foods to be fortified with vitamin B12. This includes selected soy milks, yeast spread, and vegetarian meat analogues.

The risk of toxicity from vitamin B12 intake from supplements and/or fortified foods is low. Vitamin B12 is a water soluble vitamin, and therefore any excess intake is usually excreted in the urine.

1.1.4 Causes of vitamin B12 deficiency

Table 1.2 describes causes of vitamin B12 deficiency which can be divided into four categories: nutritional deficiency, increased requirements, impaired absorption, and other gastrointestinal causes.
### Table 1.2: Causes of vitamin B12 deficiency

<table>
<thead>
<tr>
<th>Nutritional deficiency</th>
<th>Increased requirements</th>
<th>Impaired absorption</th>
<th>Other gastrointestinal causes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor intake of meats and</td>
<td>Due to pregnancy and</td>
<td>Autoimmune disease with autoantibodies against the intrinsic factor (pernicious</td>
<td>Chronic gastrointestinal symptoms e.g. dyspepsia, recurrent peptic</td>
</tr>
<tr>
<td>dairy products in the</td>
<td>lactation(21-24)</td>
<td>anaemia (25)</td>
<td>ulcer, diarrhoea(3)</td>
</tr>
<tr>
<td>elderly population (aged</td>
<td></td>
<td>Atrophic body (corpus) gastritis (due to autoantibodies to gastric parietal cells)(27)</td>
<td>Coeliac disease(31)</td>
</tr>
<tr>
<td>65 and above)(17)</td>
<td></td>
<td>Prolonged use of acid-suppression therapy or drugs(25)</td>
<td>Crohn’s disease(32)</td>
</tr>
<tr>
<td>Chronic alcoholism(18, 19)</td>
<td></td>
<td>Gastrectomy(29)</td>
<td>Fish tapeworms and other intestinal parasites(29)</td>
</tr>
<tr>
<td>Strict vegan diets(17)</td>
<td></td>
<td>or any intestinal surgery which involves gastric resection, sleeve or banding surgery(30)</td>
<td>Ileocystoplasty (i.e. a surgical reconstruction of the bladder</td>
</tr>
<tr>
<td>Malnutrition(30)</td>
<td></td>
<td></td>
<td>involving the use of an isolated segment of ileum to augment bladder</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>capacity)(33)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pancreatic failure</td>
</tr>
</tbody>
</table>

Vitamin B12 deficiency is usually the result of dietary insufficiency and is common in individuals who are strict vegetarians because vitamin B12 is only present in foods from animal origin. Because of the complex mechanism of vitamin B12 absorption, causes of malabsorption may also arise at several levels in the gastrointestinal tract. At the gastric level, the most frequent cause of significant vitamin B12 malabsorption leading to deficiency is pernicious anaemia (PA), which is an autoimmune disorder caused by the frequent presence of gastric autoantibodies directed against IF and the parietal cells. PA can affect both the elderly and young individuals.

#### 1.1.5 Diseases caused by vitamin B12 deficiency

Vitamin B12 plays an important role in DNA synthesis and neurologic function. Deficiency in vitamin B12 is associated with a wide spectrum of haematologic, neurologic and psychiatric disorders (Table 1.3) that can often be reversed by early diagnosis and prompt treatment.

#### Table 1.3: Clinical manifestations of vitamin B12 deficiency

<table>
<thead>
<tr>
<th>Haematologic(3)</th>
<th>Neurologic(38)</th>
<th>Psychiatric(38-41)</th>
<th>Cardiovascular(42, 43)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Megaloblastic anaemia</td>
<td>Paresthesias (i.e. a skin sensation such as burning or itching with no apparent physical cause)</td>
<td>Irritability, personality change</td>
<td>Possible increased risk of myocardial infarction and stroke</td>
</tr>
<tr>
<td>Pancytopenia (leukopenia, thrombocytopenia)</td>
<td>Peripheral neuropathy</td>
<td>Mild memory impairment, dementia</td>
<td></td>
</tr>
<tr>
<td>Pernicious anaemia (i.e. large immature RBCs)</td>
<td>Combined systems disease (demyelination of peripheral nerves, spinal cord, cranial nerves and the brain)</td>
<td>Depression</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Psychosis</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alzheimer’s Disease(21)</td>
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</tr>
</tbody>
</table>

#### 1.1.6 Vitamin B12 testing

Reliable and accurate assessment of vitamin B12 status is required to determine the prevalence of deficiencies of this vitamin in the Australian population and is necessary for developing suitable strategies to prevent these nutritional problems. The haematologic complications of vitamin B12 and folate deficiencies are identical. Therefore, detecting the
presence of vitamin B12 or folate deficiency, and distinguishing one from the other, depends critically on laboratory testing. These tests may be used singularly or in combination to establish the nutritional status and prevalence of deficiencies of the vitamins.

The methods used to assess vitamin B12 and folate status can either measure the:

- concentrations of the vitamins in the blood (e.g. serum vitamin B12 levels, serum or plasma folate levels); and/or
- increased levels of metabolites such as MMA and/or homocysteine.

The diagnosis of vitamin B12 deficiency has traditionally been based on measuring the total serum levels of vitamin B12. There is currently no internationally agreed definition for vitamin B12 deficiency based on clinical manifestations or on the ‘cut-off’ values that are used to define vitamin B12 deficiency, which vary between 120-200 pmol/L. However, vitamin B12 is carried on two distinct binding proteins in plasma:

- **Transcobalamin II**: binds vitamin B12 to form a complex called holotranscobalamin (holoTC). HoloTC binds only 20–30% of vitamin B12 circulating in the blood, but is responsible for delivery of vitamin B12 to cells and is considered to be the functionally important fraction, thus its name active-B12. HoloTC levels fall in vitamin B12 deficiency. Therefore, testing for this carrier protein can identify low vitamin B12 status before total serum vitamin B12 levels drop.

- **Haptocorrin**: binds the major portion of plasma vitamin B12 which is essentially inert as far as vitamin B12 delivery to cells is concerned, although it may reflect the general underlying state of vitamin B12 stores. The complex formed by the binding of haptocorrin to vitamin B12 is called HoloHC. Haptocorrin deficiency is associated with low serum vitamin B12 concentrations.

Research has shown that assays that measure holoTC-associated fraction of vitamin B12 (e.g. Axis-Shield ASA) are a more reliable indicator for identifying vitamin B12 deficiency, when used in conjunction with other available tests, such as serum MMA or homocysteine measurements. Currently available assays to measure holoTC are developed by Axis-Shield. This company recently launched a new active-B12 assay (Abbott ARCHITECT) for use in high throughput laboratories. Furthermore, elevated levels of metabolites such as MMA have been shown to be more sensitive in the diagnosis of vitamin B12 deficiency than measurement of serum B12 levels alone. Urinary MMA can be measured using high performance liquid chromatography (HPLC). Both biomarkers, holoTC and MMA, show a stronger association between low vitamin B12 concentrations and increased risk of cognitive decline and dementia in the elderly than total vitamin B12 measurements.

Table 1.4: **Comparison of the three tests used to measure vitamin B12**

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Serum/plasma B12</th>
<th>Serum holoTC</th>
<th>Serum/plasma MMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assessing intake</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>+</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Specificity</td>
<td>--</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Assessing long term and short term status of vitamin B12</td>
<td>Long term status</td>
<td>Long term and short term status</td>
<td>Long term and short term</td>
</tr>
<tr>
<td>Accepted cut-offs indicating deficient states</td>
<td>See Table 1.5</td>
<td>TC &lt;35 pmol/L</td>
<td>&gt;260 nmol/L deficient</td>
</tr>
</tbody>
</table>
Table 1.4 shows that sensitivity of serum vitamin B12 measurement for detection of vitamin B12 depletion or deficiency is good overall, but specificity is poor. The predictive value is improved when this test is combined with measurement of MMA. One study has shown that the use of a low serum vitamin B12 level as the sole means of diagnosis of vitamin B12 deficiency may miss up from 10% to 26% of patients with actual tissue B12 deficiency. The holoTC assay used on its own is also not very predictive of vitamin B12 deficiency unless it is used in conjunction with plasma MMA or with the total plasma vitamin B12. Therefore, for an accurate measure of vitamin B12 status and reserves, it is recommended that serum vitamin B12 levels are combined with a measure of a metabolic marker of vitamin B12 reserves such as MMA, holoTC or homocysteine.

1.1.7 Serum vitamin B12 target values

The cut-off value for vitamin B12 deficiency varies markedly between laboratories worldwide. Table 1.5 presents the “usual or approximate” reference intervals for vitamin B12 deficiency.

Table 1.5: Vitamin B12 reference intervals

<table>
<thead>
<tr>
<th>Status</th>
<th>Vitamin B12 levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal range</td>
<td>200-900 pg/ml† (130-850 pmol/L)</td>
</tr>
<tr>
<td>Deficient</td>
<td>&lt; 200* pg/ml (&lt; 130 pmol/L)</td>
</tr>
</tbody>
</table>

* This is an unsafe range as many in the population exhibit neurological symptoms of deficiency at much higher concentrations. The lowest concentration to be considered normal is 221 pmol/L. † pmol/L = 0.738 x pg/ml

As discussed earlier, elevated homocysteine levels can be a useful indicator for vitamin B12 deficiency, because serum homocysteine levels increase as vitamin B12 stores fall. Serum homocysteine levels greater than 9 µmol/L suggest the beginning of depleted vitamin B12 reserves and levels greater than 15 µmol/L is indicative of depleted vitamin B12 reserves. However, caution should be taken with this test as homocysteine levels may also increase with folate deficiency.

It is important to distinguish between low vitamin B12 status (defined as subclinical cobalamin deficiency (SCCD)) and outright vitamin B12 deficiency. Low vitamin B12 status denotes a condition in which laboratory tests indicate depletion of vitamin B12 stores as judged by being outside of the normal reference range. In the case of direct measures of vitamin B12 [serum vitamin B12 or holotranscobalamin (holoTC)], low vitamin B12 status is indicated by being below the lower limit of the reference range (for vitamin B12 < 200 pg/mL or <148 pmol/L; for holoTC <35 pmol/L), whereas for indirect measures of metabolites (MMA or homocysteine), low vitamin B12 status would be indicated by a level above the upper limit of the reference range (for MMA >260 nmol/L; for homocysteine, > 12 µmol/L).

There are large numbers of individuals with low vitamin B12 status who do not progress to outright deficiency. This may be attributed to the degree of impairment of the process of assimilation and absorption of vitamin B12 in relation to the daily requirement for the vitamin. Complete abrogation of physiologic vitamin B12 absorption, such as occurs after total gastrectomy, ileal resection, or advanced autoimmune pernicious anaemia, will inexorably lead to a degree of depletion of the vitamin that can no longer sustain cellular requirements and that would, with time, lead to both functional and structural abnormalities. However, in the food vitamin B12 malabsorption states, the basic mechanism of intrinsic factor-dependent vitamin B12 absorption remains intact, but some aspect of the assimilative process is impaired, as in non-immune atrophic gastritis or with the chronic use of proton
There is uncertainty and ongoing debate as to whether low vitamin B12 status per se may be associated with subtle degrees of deficiency that have consequences of public health significance.\(^{(44)}\)

### 1.1.8 Prevalence of vitamin B12 deficiency in Australia

The true prevalence of vitamin B12 deficiency in the general Australian population remains unknown. The incidence appears to increase with age (\(>65\) years) and with the ubiquitous use of gastric acid–blocking agents.\(^{(66)}\) An Australian study published in 2012 found 14% of 130 patients living in residential aged care facilities in southern Tasmania were vitamin B12 deficient, defined as serum vitamin B12 levels less than 150 pmol/L.\(^{(67)}\) Another study published in 2006 examined the prevalence of low serum vitamin B12 in a representative sample of 3,508 persons aged 50+ years between 1997 and 2000.\(^{(68)}\) Low serum vitamin B12 (defined as \(< 185\) pmol/L) was found in 22.9% of participants.

### 1.1.9 Service providers claiming MBS benefits for vitamin B12 testing

Most pathology in Australia is provided in comprehensive laboratories that provide a wide range of testing services at a single location. Only approved pathology practitioners are eligible to claim vitamin B12 testing.

### 1.2 The clinical flowcharts

The clinical decision pathway that determines whether vitamin B12 testing should be undertaken is provided in Figure 1.3.
Figure 1.3: Clinical flow chart for vitamin B12 testing

Patient presents to clinician (e.g. General Practitioner, Obstetrician etc)

Does the patient have any of the following clinical symptoms of vitamin B12 deficiency?
- Neuromotor symptoms including:
  - Paresthesia; or
  - Ataxia; or
  - Decreased reflexes; or
  - Restless leg syndrome; or
  - Peripheral neuropathy.
- Neuropsychiatric symptoms including:
  - Dementia
  - Depression
  - Psychosis
  - Personality changes

Does the patient have any of the following risk factors associated with vitamin B12 deficiency?
- Vegetarians
- Patients >65
- Institutionalised patients or patients in aged care facilities
- Newborn children of vegetarian or malnourished mothers
- Gastric surgery patients
- Atrophic gastritis patients
- H. Pylori infected patients
- Patients with gastrointestinal disorders e.g. Crohn’s, Coeliac disease

Does the patient have any of the following haematological symptoms of vitamin B12 deficiency?
- Anemia
- Macrocytic anaemia
- Macrocytosis
- Pernicious anaemia

Neuromotor symptoms including:
- Paresthesia; or
- Ataxia; or
- Decreased reflexes; or
- Restless leg syndrome; or
- Peripheral neuropathy.

Neuropsychiatric symptoms including:
- Dementia
- Depression
- Psychosis
- Personality changes

Does the patient have any of the following clinical symptoms of vitamin B12 deficiency?
- Yes
- No

Patient ineligible to claim benefits under MBS item numbers 66599 or 66602

Is Vitamin B12/folate testing clinically relevant?
- Yes
- No

Measure Vitamin B12 and claim MBS item number 66599

Is Vitamin B12/folate testing clinically relevant?
- Yes
- No

Patient ineligible to claim benefits under MBS item numbers 66599 or 66602

Measure Vitamin B12 and/or folate and claim MBS item number 66602
2 REVIEW METHODOLOGY

The review methodology comprises an analysis of secondary data (e.g. MBS claims), a guideline concordance analysis, and a systematic literature review for clinical and economic evidence. This Chapter presents clinical research questions and the methodology used for each of these review components.

2.1 Secondary data analysis

Data from Medicare Australia were analysed to determine whether the existing MBS item numbers for vitamin B12 testing (66599 and 66602) are appropriate.

2.1.1 The research questions for the MBS analysis

The MBS data were examined to determine:

(1) Whether the existing MBS items for service (66599 and 66602), including the associated explanatory notes, are appropriate
   a. How frequent are the MBS item numbers under review claimed?
   b. Are there any age, sex, temporal or geographic trends associated with usage of these item numbers?
   c. Are the Medicare claims data consistent with trends in the incidence/prevalence of the conditions/diseases being addressed by the services?
   d. What is the frequency of vitamin B12 testing per patient?
   e. What is the frequency of vitamin B12 testing by referring clinician?
   f. What is the profile of referring clinician for vitamin B12 testing?

2.1.2 Methods for analysis of MBS data

MBS data relates to private medical services (provided in- or out-of-hospital), where the services are provided to patients regardless of whether or not they have private health cover. MBS in-hospital services are mainly provided in private hospitals and day surgery clinics, but patients can elect to be treated as a private patient in a public hospital.

MBS data were analysed by patient gender, age group, patterns of use and discipline of provider requesting the test.

Results of the analysis of the MBS data is presented in Chapter 3.

2.2 Guideline concordance

2.2.1 The research questions for the guideline concordance analysis

The research questions addressed as part of the Review using guideline concordance analysis are:

(1) Are the existing MBS items for service consistent with evidence-based (or in the absence of evidence, consensus-based) recommendations provided in relevant clinical practice guidelines?
(2) What are the appropriate clinical indications for vitamin B12 testing?
(3) How frequently should vitamin B12 levels be tested?
   a. in apparently healthy populations (including pregnant women, elderly, vegetarians)?
   b. in patients with chronic disease linked to vitamin B12 deficiency (e.g. infants with metabolic disease; patients with anaemia or haematologic, neurologic, psychiatric, gastrointestinal and malabsorption disorders)?

2.2.2 Methods for guideline concordance analysis

Searches of guidelines databases and relevant discipline websites were undertaken to locate any existing guidelines relevant to vitamin B12 testing. Analysis of MBS item numbers 66599 and 66602 was undertaken relative to ‘best practice’, as recommended in relevant Australian clinical practice guidelines. Where Australian clinical practice guidelines do not exist, other guidelines in operation in comparable health systems overseas were included. Where guidelines existed, they were assessed for quality using the AGREE II instrument. Differences in the purpose and intended audience of any such guidelines were considered, documented and acknowledged.

See Chapter 4 for results of the concordance analysis for vitamin B12 testing.

2.3 Systematic literature review for clinical evidence

2.3.1 The clinical questions for the systematic literature review

The clinical questions that were the focus of the literature review are:

(1) What are the appropriate clinical indications for vitamin B12 testing?
(2) Is there evidence that testing for vitamin B12 levels improves health outcomes?
   a. in apparently healthy populations (including pregnant women, elderly, vegetarians)?
   b. in patients with chronic disease linked to vitamin B12 deficiency?
(3) Are there risks/harms associated with vitamin B12 testing?
(4) Does quality of testing vary according to testing platform?

2.3.2 Search strategy

A comprehensive search of peer-reviewed scientific literature was conducted to identify relevant studies addressing the key questions. Electronic databases were searched for original research papers including systematic reviews as shown in Table 2.1. Searches were restricted to studies published in the English language between January 2006 and September 2013. Databases maintained by Health Technology Assessment (HTA) agencies were searched to identify existing assessments of vitamin B12 testing.

Table 2.1: Databases searched

<table>
<thead>
<tr>
<th>Database</th>
<th>Search period</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEDLINE</td>
<td>2006 – September 2013</td>
</tr>
<tr>
<td>PubMed</td>
<td>2006 – September 2013</td>
</tr>
<tr>
<td>The Cochrane Library (includes Cochrane Database of Systematic Reviews, Database of Abstracts of Reviews of Effects, Cochrane Central Register of Controlled Trials, NHS Economic Evaluation Database, Health Technology Assessment, Cochrane Methodology Register)</td>
<td>2006 – September 2013</td>
</tr>
<tr>
<td>Relevant HTA websites and databases&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Up to September 2013</td>
</tr>
</tbody>
</table>

Reference lists of systematic, semi-systematic and selected narrative reviews were also reviewed. In addition, during the consultation process clinicians were asked if they were aware of any relevant clinical guidelines, unpublished studies or reviews relevant to this review of vitamin B12 testing.

2.3.3 Eligibility criteria for studies

The PICO (Population, Intervention, Comparator, Outcomes) criteria<sup>70</sup> was used to develop well-defined questions for the search of published literature. This involved focusing the question on four elements:

- the target population for the intervention;
- the intervention being considered;
- the comparator for the existing MBS service (where relevant); and
- the clinical outcomes that are most relevant to assess safety and effectiveness.

The PICO criteria were determined on the basis of information provided in the literature, as well as clinical advice. The PICO criteria for the review of vitamin B12 testing are shown in Table 2.2.

---

<sup>3</sup> The following HTA websites were searched: Agency for Healthcare Research and Quality (AHRQ) at www.ahrq.gov; Canadian Agency for Drugs and Technologies in Health (CADTH) at http://www.cadth.ca/en; National Institute for Health and Care Excellence (NICE) at www.nice.org.uk; Australasian College of Surgeons (ASERNIP-S) at http://www.surgeons.org/for-health-professionals/audits-and-surgical-research/aser nip-s/
Table 2.2: PICO criteria for the vitamin B12 testing items under review

<table>
<thead>
<tr>
<th>Population</th>
<th>Intervention</th>
<th>Comparator</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients at risk of vitamin B12 deficiency, including (but not limited to):</td>
<td>Vitamin B12</td>
<td>No testing</td>
<td>Effectiveness</td>
</tr>
<tr>
<td>(1) Pregnant women</td>
<td>testing</td>
<td></td>
<td>• Physical health outcomes as a consequence of vitamin B12 testing</td>
</tr>
<tr>
<td>(2) Elderly</td>
<td></td>
<td></td>
<td>(e.g. all-cause mortality, anaemia, NTDs, CVD, neuropathy, depression</td>
</tr>
<tr>
<td>(3) Alcoholics</td>
<td></td>
<td></td>
<td>and dementia)</td>
</tr>
<tr>
<td>(4) Vegans</td>
<td></td>
<td></td>
<td>Safety</td>
</tr>
<tr>
<td>(5) Patients with gastrointestinal and malabsorption disorders</td>
<td></td>
<td></td>
<td>• Complications associated with vitamin B12 testing</td>
</tr>
<tr>
<td>(6) Infants with metabolic disease</td>
<td></td>
<td></td>
<td>(e.g. infection, needle injuries)</td>
</tr>
<tr>
<td>(7) Patients with anaemia and haematologic diseases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(8) Patients with neurologic disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(9) Patients with psychiatric disorders</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The detailed search strategy and terms used is presented in Appendix 4.

Studies were excluded on the basis of citation information and/or abstract, where it was obvious that they did not meet the inclusion criteria. Where there was any doubt about any reference based on the title and/or abstract, the full paper was retrieved and evaluated. Table 2.3 lists the pre-specified inclusion and exclusion criteria.

Table 2.3: Inclusion/exclusion criteria for identification of relevant studies

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Search period</td>
<td>2006 – 2013</td>
</tr>
<tr>
<td></td>
<td>Should there be limited data available during this period, the search will be extended back in five year increments until sufficient data are sourced.</td>
</tr>
<tr>
<td>Publication type</td>
<td>Clinical studies included. Non-systematic reviews, letters, editorials, animal, in vitro and laboratory studies excluded.</td>
</tr>
<tr>
<td></td>
<td><strong>Systematic reviews</strong></td>
</tr>
<tr>
<td></td>
<td>Systematic reviews that have been superseded were excluded</td>
</tr>
<tr>
<td></td>
<td><strong>Primary studies</strong></td>
</tr>
<tr>
<td></td>
<td>Primary studies published during the search period of included systematic reviews were excluded</td>
</tr>
<tr>
<td></td>
<td><strong>Effectiveness studies</strong> included if:</td>
</tr>
<tr>
<td></td>
<td>• prospective, comparative trial</td>
</tr>
<tr>
<td></td>
<td>• &gt;20 patients</td>
</tr>
<tr>
<td></td>
<td><strong>Safety studies</strong> included if:</td>
</tr>
<tr>
<td></td>
<td>• &gt;50 patients</td>
</tr>
<tr>
<td>Intervention</td>
<td>Vitamin B12 testing</td>
</tr>
<tr>
<td>Comparator</td>
<td>No vitamin B12 testing</td>
</tr>
<tr>
<td>Outcome</td>
<td>Studies must report on at least one of the following outcomes:</td>
</tr>
<tr>
<td></td>
<td>• Patient outcomes: morbidity, mortality, quality of life</td>
</tr>
<tr>
<td></td>
<td>• Safety: adverse physical health outcomes or complications associated with testing</td>
</tr>
<tr>
<td>Language</td>
<td>Non-English language articles excluded</td>
</tr>
</tbody>
</table>
2.3.4 Process for classifying the evidence
All eligible studies were assessed according to the National Health and Medical Research Council (NHMRC) Dimensions of Evidence (refer to Appendix 5). There are three main domains: strength of the evidence, size of the effect, and relevance of the evidence. One aspect of the ‘strength of the evidence’ domain is the level of evidence, which is assigned using the NHMRC Levels of Evidence (Appendix 5). For any eligible publications, study quality was evaluated and reported using the NHMRC Quality Criteria (Appendix 5) for randomised controlled trials (RCTs), cohort studies, case-control studies and systematic reviews.

The results of the review of clinical evidence for vitamin B12 testing are presented in Chapter 5.

2.4 Systematic literature review for economic evidence
The research question for the review of economic literature is:

(1) What is the evidence regarding the cost implications associated with vitamin B12 testing compared with not testing?

Consistent with the terms of reference, a formal modelled economic evaluation of lipectomy was not in-scope. The review relied on published costing studies and economic analyses identified through a systematic literature search of the databases shown in Table 2.1. The detailed search strategy and terms used are presented in Appendix 4. Citations were reviewed to identify acceptable evidence including: trial-based costing studies, cost analyses and economic modelling studies. Acceptable outcomes were limited to: cost, incremental cost-effectiveness ratio (e.g. cost per event avoided, cost per life year gained, cost per quality adjusted life year or disability adjusted life year).

The results of the search for economic evaluations of vitamin B12 testing are presented in Chapter 6.
3 SECONDARY DATA ANALYSIS

This Chapter presents an analysis of the available secondary data (including MBS data) that describes the use of vitamin B12 testing in Australia. When interpreting the data, it is important to keep in mind that the MBS item numbers within scope are for both vitamin B12 and folate testing. It is not possible to separate the data that specifically relates to vitamin B12 testing alone.

3.1 MBS item number usage and expenditure

Figure 3.1 shows the number of claims for each of the MBS vitamin B12/folate testing items over the past 10 years. The number of claims for MBS item 66599 has more than doubled (+119%) from 282,531 in 2003/04 to 618,744 in 2012/13. Over the same timeframe the number of claims for MBS item 66602 has had an even greater increase (+307%) from 522,980 to 2,129,051. The total number of claims for both items has increased over the past ten years from 0.98m to 2.7m.

Figure 3.1: Number of claims for MBS items 66599 and 66602, 2003/04 to 2012/13

![Number of MBS item claims](image)

Source: Department of Human Services – Medicare Australia

Figure 3.2 shows the benefits paid for MBS items 66602 and 66599 over the past ten years by state. The increase in benefits paid for both items reflects the increase in claims. Benefits paid for MBS item 66599 increased from $5.7m to $12.5m (+120%) whilst benefits for MBS item 66602 increased from $19.2m to $78.5m (+309%). Whilst total benefits increased significantly, the proportion of benefits paid to each state and territory remained relatively constant over the ten-year period.
Figure 3.2: Benefits paid for MBS items 66599 and 66602 by state and territory, 2003/04 to 2012/13

Figure 3.3 shows that the highest proportion of benefits paid over the past ten years was in New South Wales (NSW) (34% for 66599 and 38% of total for 66602). This was followed by Victoria (30% of total for 66599 and 28% of total for 66602). For MBS item 66602, Queensland had the third highest proportion of claims (19%) followed by Western Australia (8%) and South Australia (5%). This pattern was slightly different for MBS item 66599, with South Australia having the third highest proportion of claims (13%) followed by Queensland (11%) and Western Australia (8%).
To further explore geographical trends in testing, an analysis was conducted of the number of services per capita (i.e. per 100,000 population), according to the address at the time of claiming of the patient to whom the service was rendered. In 2012/13, there were 2,666 claims per 100,000 people enrolled in Medicare across Australia for item 66599 and 9,172 claims per 100,000 people for item 66602 (Table 3.1). South Australia had the highest rate of claiming for item 66599 per capita (3,635 claims per 100,000 population), followed by the Australian Capital Territory (ACT) and NSW. The lowest per capita rate of claims for item 66599 was in the Northern Territory (762 claims per 100,000 population). For item 66602, the highest number of claims per capita in 2012/13 was for NSW and Victoria (over 10,000 claims per 100,000 population in both states), while Tasmania had the lowest (less than 4,000 claims per 100,000 population).

Table 3.1: Claims for MBS items 66599 and 66602 per capita (100,000 population)*, 2008/09 to 2012/13

<table>
<thead>
<tr>
<th>State/territory</th>
<th>Item 66599</th>
<th>Item 66602</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSW</td>
<td>1,922</td>
<td>2,065</td>
</tr>
<tr>
<td>VIC</td>
<td>1,928</td>
<td>2,052</td>
</tr>
<tr>
<td>QLD</td>
<td>1,013</td>
<td>961</td>
</tr>
<tr>
<td>SA</td>
<td>3,034</td>
<td>3,172</td>
</tr>
<tr>
<td>WA</td>
<td>1,525</td>
<td>1,165</td>
</tr>
<tr>
<td>TAS</td>
<td>1,910</td>
<td>2,204</td>
</tr>
<tr>
<td>ACT</td>
<td>2,163</td>
<td>2,285</td>
</tr>
<tr>
<td>NT</td>
<td>420</td>
<td>555</td>
</tr>
<tr>
<td>Total</td>
<td>1,773</td>
<td>1,822</td>
</tr>
</tbody>
</table>

Source: Department of Human Services – Medicare Australia
* Services per capita (i.e. per 100,000 population) is calculated by dividing the number of services processed in a month by the number of people enrolled in Medicare at the end of that month.

Data relating to the average fee per service and average benefit per service from 2008/09 to 2012/13 are summarised in Table 3.2. The proportion of services bulk billed was high (more than 94% of services) from 2008/09 to 2012/13, which is consistent with the high proportion of out-of-hospital services (approximately 97%).
Table 3.2: Fees charged and benefits paid for MBS items 66599 and 66602, 2008/09 to 2012/13

<table>
<thead>
<tr>
<th>Item</th>
<th>2008/09</th>
<th>2009/10</th>
<th>2010/11</th>
<th>2011/12</th>
<th>2012/13</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of services</td>
<td>382,241</td>
<td>399,282</td>
<td>447,211</td>
<td>520,688</td>
<td>618,744</td>
<td>2,368,166</td>
</tr>
<tr>
<td>66599</td>
<td>382,241</td>
<td>399,282</td>
<td>447,211</td>
<td>520,688</td>
<td>618,744</td>
<td>2,368,166</td>
</tr>
<tr>
<td>66602</td>
<td>1,476,465</td>
<td>1,586,968</td>
<td>1,667,155</td>
<td>1,821,490</td>
<td>2,129,051</td>
<td>8,681,129</td>
</tr>
<tr>
<td>Total fees charged</td>
<td>$7,995,258</td>
<td>$8,310,784</td>
<td>$9,225,951</td>
<td>$10,698,027</td>
<td>$12,665,119</td>
<td>$48,895,138</td>
</tr>
<tr>
<td>66599</td>
<td>$7,995,258</td>
<td>$8,310,784</td>
<td>$9,225,951</td>
<td>$10,698,027</td>
<td>$12,665,119</td>
<td>$48,895,138</td>
</tr>
<tr>
<td>66602</td>
<td>$56,002,248</td>
<td>$60,132,026</td>
<td>$62,715,325</td>
<td>$68,264,085</td>
<td>$79,337,023</td>
<td>$326,450,707</td>
</tr>
<tr>
<td>Average fee per service</td>
<td>$20.91</td>
<td>$20.81</td>
<td>$20.63</td>
<td>$20.54</td>
<td>$20.46</td>
<td>$20.64</td>
</tr>
<tr>
<td>66599</td>
<td>$20.91</td>
<td>$20.81</td>
<td>$20.63</td>
<td>$20.54</td>
<td>$20.46</td>
<td>$20.64</td>
</tr>
<tr>
<td>66602</td>
<td>$37.93</td>
<td>$37.89</td>
<td>$37.62</td>
<td>$37.48</td>
<td>$37.26</td>
<td>$37.60</td>
</tr>
<tr>
<td>Total benefits paid</td>
<td>$7,843,976</td>
<td>$8,102,717</td>
<td>$9,061,322</td>
<td>$10,545,169</td>
<td>$12,484,776</td>
<td>$48,037,960</td>
</tr>
<tr>
<td>66599</td>
<td>$7,843,976</td>
<td>$8,102,717</td>
<td>$9,061,322</td>
<td>$10,545,169</td>
<td>$12,484,776</td>
<td>$48,037,960</td>
</tr>
<tr>
<td>66602</td>
<td>$55,208,644</td>
<td>$58,728,189</td>
<td>$61,669,203</td>
<td>$67,358,102</td>
<td>$78,506,111</td>
<td>$321,470,248</td>
</tr>
<tr>
<td>Average benefit per service</td>
<td>$20.52</td>
<td>$20.29</td>
<td>$20.26</td>
<td>$20.25</td>
<td>$20.17</td>
<td>$20.28</td>
</tr>
<tr>
<td>66599</td>
<td>$20.52</td>
<td>$20.29</td>
<td>$20.26</td>
<td>$20.25</td>
<td>$20.17</td>
<td>$20.28</td>
</tr>
<tr>
<td>66602</td>
<td>$37.39</td>
<td>$37.00</td>
<td>$36.99</td>
<td>$36.97</td>
<td>$36.87</td>
<td>$37.03</td>
</tr>
<tr>
<td>66599</td>
<td>$9.42%</td>
<td>$9.43%</td>
<td>$9.58%</td>
<td>$9.60%</td>
<td>$9.71%</td>
<td>$9.54%</td>
</tr>
<tr>
<td>66602</td>
<td>$9.42%</td>
<td>$9.43%</td>
<td>$9.58%</td>
<td>$9.60%</td>
<td>$9.71%</td>
<td>$9.54%</td>
</tr>
<tr>
<td>Average OOP cost*</td>
<td>$9.14</td>
<td>$12.33</td>
<td>$13.00</td>
<td>$9.92</td>
<td>$10.26</td>
<td>$10.96</td>
</tr>
<tr>
<td>66599</td>
<td>$9.42%</td>
<td>$9.43%</td>
<td>$9.58%</td>
<td>$9.60%</td>
<td>$9.71%</td>
<td>$9.54%</td>
</tr>
<tr>
<td>66602</td>
<td>$9.42%</td>
<td>$9.43%</td>
<td>$9.58%</td>
<td>$9.60%</td>
<td>$9.71%</td>
<td>$9.54%</td>
</tr>
</tbody>
</table>

Source: Department of Human Services – Medicare Australia  
*Average out-of-pocket cost is equal to ‘fees charged for patient-billed out-of-hospital services’ minus ‘benefits paid for patient-billed out-of-hospital services’ divided by ‘number of patient-billed out-of-hospital services’

### 3.2 Age and gender profile of patients

The pattern of use by age and gender for item numbers 66599 and 66602 is shown in Figure 3.4. Vitamin B12/folate testing claimed under MBS item numbers 66599 and 66602 is performed for both males and females; however, on average (across all age groups), the test is undertaken more frequently in females than males (1.8 times more frequently for MBS item 66599 and 1.9 times more frequently for MBS item 66602). For both MBS items, this difference is greatest in the 15-24 and 25-34 year age groups where females are over 3 times more likely to have a vitamin B12/folate test.

For both males and females, the number of tests being performed is higher in older age groups, particularly for MBS item 66599. This trend was slightly greater for males, with 76% of claims for MBS item 66599 being for those aged 45 years and over versus 64% for females. Similarly, for MBS item 66602, 71% of claims for males were aged 45 years and over versus 60% for females.
3.3 Frequency of testing by patient

An analysis of vitamin B12/folate testing frequency per patient was conducted. For item 66599, there was an increase in the overall number of patients tested for vitamin B12/folate, from 350,954 patients in 2008/09 to 502,547 patients in 2012/13 (+43%). As can be clearly seen from Figure 3.5, over the period 2008/09 to 2012/13 there has been very little change in the proportion of patients receiving either one test per year (92%), two tests per year (7.5%), or three or more tests per year (0.8%).

For item 66602, there was an increase in the overall number of patients tested for vitamin B12/folate, from 1,324,646 in 2008/09 to 1,706,258 in 2012/13 (+29%). The proportion of patients receiving only one test within a year was stable over the five-year timeframe (90%). Approximately 9% of patients received two tests per year and approximately 1% received three or more tests per year.

These data suggest that the majority of vitamin B12/folate testing services are being undertaken for the purposes of screening/testing rather than monitoring.

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Figure 3.5: Use of the MBS items for vitamin B12/folate testing by age and gender (2008/09 to 2012/13)

Source: Department of Human Services – Medicare Australia

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4 Based on data processed to 31 May 2013; 2012/13 is therefore incomplete.
3.4 Profile of providers requesting vitamin B12/folate testing services

The profile of providers requesting vitamin B12/folate services was examined over time from 2008/09 to 2012/13 (Table 3.3). Over the five-year time period, there were no material changes in the pattern of requesting providers. General practitioners (GPs) and other medical practitioners (OMPs) accounted for approximately 71% of all providers requesting vitamin B12/folate testing services for item 66599. Internal medicine consultant physicians accounted for approximately 14% of all provider counts over the five-year time period, followed by general surgeons (specialist – subspecialties) and interns. There was a large variety of other providers requesting services, but they each accounted for less than 2% of provider counts.

The profile of providers requesting item 66602 was similar to that of item 66599. GPs and OMPs accounted for 67% of all providers requesting item 66602, followed by internal medicine consultant physicians (approximately 14%), psychiatrists, general surgeons (specialist – subspecialties) and interns.

Table 3.3: Number of providers requesting MBS items 66599 and 66602, 2008/09 to 2012/13

Source: Department of Human Services – Medicare Australia
* Data are incomplete for 2008/09 and 2009/10 because small provider counts were suppressed.
* Based on data processed to 31 May 2013; 2012/13 is therefore incomplete.

Other includes the following peer groups, which each accounted for <1% of the provider count: Dermatologist – specialist; Surgeon – non-specialist; Other medical specialist; IVF; Unclassified-miscellaneous-non-specialist; Anaesthesists – specialist; Dentist/orthodontist; Therapeutic radiologist/therapeutic nuclear medicine – specialist; Pathologist; Anaesthetics – non-specialist; Specialist physician – internal
3.5 Frequency of requests for testing by provider

An analysis was conducted of requests for item 66599 and 66602 by frequency from any one provider. For both items, there was an increase over the period 2008/09 to 2012/13 in the overall number of providers requesting vitamin B12/folate testing. For item 66599, the majority of providers (97%) requested 100 or fewer tests per year. Approximately 2% of providers requested 101 to 200 tests per year and the remaining providers requested more than 200 tests per year (Table 3.4). There were a small number of providers (ranging from 36 to 62 per year) that requested more than 400 tests per year.

From 2008/09 to 2012/13, approximately 88% of the providers requesting item 66602 requested 100 or fewer tests per year (Table 3.4). Approximately 7% of providers requested between 101 and 200 tests per year and 2.5% requested 201 to 300 tests per year. Between 2010/11 and 2012/13, over 500 providers requested more than 400 tests per year.
Table 3.4: Frequency of MBS items 66599 and 66602 requested per year by any provider, 2008/09 to 2012/13*

<table>
<thead>
<tr>
<th>No. of tests requested in the year</th>
<th>2008/09</th>
<th>2009/10</th>
<th>2010/11</th>
<th>2011/12</th>
<th>2012/13</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Item 66599</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-100</td>
<td>26,341</td>
<td>26,521</td>
<td>26,266</td>
<td>32,801</td>
<td>34,602</td>
<td>198,312</td>
</tr>
<tr>
<td>101-200</td>
<td>461</td>
<td>459</td>
<td>579</td>
<td>672</td>
<td>724</td>
<td>2,851</td>
</tr>
<tr>
<td>201-300</td>
<td>122</td>
<td>133</td>
<td>172</td>
<td>172</td>
<td>182</td>
<td>733</td>
</tr>
<tr>
<td>301-400</td>
<td>34</td>
<td>46</td>
<td>46</td>
<td>45</td>
<td>45</td>
<td>251</td>
</tr>
<tr>
<td>Total</td>
<td>27,586</td>
<td>31,058</td>
<td>33,551</td>
<td>34,857</td>
<td>34,530</td>
<td>183,530</td>
</tr>
</tbody>
</table>

| Item 66602                        |         |         |         |         |         |       |
| 1-100                             | 31,477  | 31,263  | 33,131  | 33,401  | 34,813  | 167,896 |
| 101-200                           | 2,138   | 2,390   | 2,537   | 2,765   | 2,917   | 12,800 |
| 201-300                           | 772     | 821     | 912     | 1,022   | 1,128   | 4,377  |
| 301-400                           | 380     | 420     | 465     | 510     | 541     | 2,856  |
| Total                             | 34,590  | 36,303  | 38,231  | 40,232  | 41,162  | 190,205 |

Source: Department of Human Services – Medicare Australia
* Based on data processed to 31 May 2013; 2012/13 is therefore incomplete.
4 REVIEW OF GUIDELINES RELEVANT TO VITAMIN B12 TESTING

This Chapter presents the results of the literature search for clinical practice guidelines relevant to vitamin B12 testing.

4.1 Clinical practice guidelines

4.1.1 Australian Guidelines
No Australian guidelines were identified that made recommendations on vitamin B12 testing.

4.1.2 International Guidelines
International guidelines related to the use of vitamin B12 vary widely in their recommendations. While some recommend vitamin B12 testing as a screening tool in commonly encountered illnesses such as dementia, others suggest restricting testing to patients who have already undergone pre-test investigations (such as full blood examinations and blood film examination).

A rapid review from the Division of Evidence Development and Standards at Health Quality Ontario (see Chapter 5.1 for further details) identified three guidelines on the diagnosis of vitamin B12 deficiency. The three guidelines were assessed using the AGREE appraisal tool. The recommendations from each of the guidelines are listed in Table 4.1. Two of the guidelines were systematic reviews with recommendations. The guideline by the British Columbia Medical Association and Ministry of Health was not explicitly based on a systematic review of the literature. In the methods that are reported on the website that published the guideline, the authors state that a full systematic review may not be conducted for all of their guidelines. As a result, it is unclear whether this guideline was based on the results of a systematic review. The three guidelines scored poorly on linking the evidence to the recommendations.

A further nine guidelines, eight of which focused on specific clinical conditions or patient populations, were identified in the literature search for clinical practice guidelines. Relevant recommendations from these guidelines are summarised in Table 4.1.

Table 4.1: Guidelines relating to vitamin B12 testing

<table>
<thead>
<tr>
<th>Title</th>
<th>Author and country</th>
<th>Populations</th>
<th>Frequency</th>
<th>Overall recommendation</th>
</tr>
</thead>
</table>
| Cobalamin (vitamin B12) Deficiency - Investigation and Management (2012) | British Columbia Medical Association/ Ministry of Health, Canada | - Patients with unexplained neurologic symptoms (parasthesia, numbness, poor motor coordination, memory lapses)  
- Patients with macrocytic anaemia or macrocytosis  
- Other populations where testing is considered include:  
o Elderly >75 years  
o Inflammatory bowel disease (of small intestine)  
o Gastric or small intestine resection  
o Prolonged vegan diet (no | Not reported | Routine screening for vitamin B12 deficiency is not recommended |
<table>
<thead>
<tr>
<th>Title</th>
<th>Author and country</th>
<th>Populations</th>
<th>Frequency</th>
<th>Overall recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Best practice in primary care pathology: review 1 (2005)(^73)</td>
<td>Smellie et al, UK</td>
<td>● Patients with macrocytic anaemia&lt;br&gt;● Patients with macrocytosis&lt;br&gt;● Patients with specific neuropsychiatric abnormalities</td>
<td></td>
<td>Emphasise the importance of attempting to assess whether deficiency is present before requesting vitamin B12 measurement unless lack of compliance is suspected or anaemia recurs</td>
</tr>
<tr>
<td>Vitamin B12 (cobalamin) deficiency in elderly patients (2004)(^72)</td>
<td>Andres et al, Canada</td>
<td>● All elderly who are malnourished&lt;br&gt;● All patients in institutions and psychiatric hospitals&lt;br&gt;● All patients with haematological or neuropsychiatric manifestations of vitamin B12 deficiency</td>
<td>Not reported</td>
<td>All people over 65 years of age who are malnourished, all people in institutions or psychiatric hospitals, and all people with haematological or neuropsychiatric manifestations of vitamin B12 deficiency should have their serum vitamin B12 levels measured</td>
</tr>
<tr>
<td>Guidelines on the investigation and diagnosis of cobalamin and folate deficiencies (1994)(^76)</td>
<td>The British Committee for Standards in Haematology (BCSH) UK</td>
<td>Patients with the following clinical indications:&lt;br&gt;● Gastrointestinal disease&lt;br&gt;● Neurological disease&lt;br&gt;● Psychiatric disorders&lt;br&gt;● Malnutrition&lt;br&gt;● Alcohol abuse&lt;br&gt;● Autoimmune disease of the thyroid, adrenal and parathyroid glands&lt;br&gt;● Family history of pernicious anaemia&lt;br&gt;● Infertility&lt;br&gt;● Haematological diseases associated with vitamin deficiency&lt;br&gt;● Drugs that interfere with vitamin absorption&lt;br&gt;● Metabolic disease in infants</td>
<td>Not reported</td>
<td>Serum B12 testing (and serum and red cell folate testing) follow the initial investigation of blood count and blood film examination&lt;br&gt;MMA and Hcy measurements are considered as “subsidiary investigations”</td>
</tr>
<tr>
<td>Chronic kidney Disease (CKD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical Practice Kidney Disease</td>
<td></td>
<td>Patients with CKD</td>
<td>Not reported</td>
<td>Vitamin B12 testing</td>
</tr>
<tr>
<td>Title</td>
<td>Author and country</td>
<td>Populations</td>
<td>Frequency</td>
<td>Overall recommendation</td>
</tr>
<tr>
<td>----------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------------------</td>
<td>--------------------------------------------</td>
<td>-----------</td>
<td>------------------------</td>
</tr>
<tr>
<td>Guideline for anaemia in chronic kidney disease (2012)(^{(77)})</td>
<td>Improving Global Outcomes (KDIGO) International(^{(5)})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dementia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supporting people with dementia and their carers in health and social care (2012)(^{(78)})</td>
<td>National Institute for Health and Care Excellence (NICE) UK</td>
<td>Patients presenting with dementia symptoms</td>
<td>Not reported</td>
<td>Serum vitamin B12 (and folate) is recommended as a basic dementia screen to be performed at the time of presentation, usually within primary care</td>
</tr>
<tr>
<td>Practical Guidelines for the Recognition and Diagnosis of Dementia (2012)(^{(79)})</td>
<td>Galvin and Sadowsky US</td>
<td>Patients presenting with dementia symptoms</td>
<td>Not reported</td>
<td>Testing serum vitamin B12 is one of the pre-diagnostic tests to determine coexisting disorders</td>
</tr>
<tr>
<td>Cognitive impairment in the elderly-Recognition, diagnosis and management (2007)(^{(80)})</td>
<td>British Columbia Medical Association/Ministry of Health, Canada</td>
<td>Elderly with cognitive impairment and dementia</td>
<td>Not reported</td>
<td>Vitamin B12 testing is recommended as one of the laboratory tests in the initial work-up of suspected dementia or mild cognitive impairment</td>
</tr>
<tr>
<td>A synopsis of the practice parameters on dementia from the American academy of neurology on the diagnosis of dementia (2004)(^{(81)})</td>
<td>Pittner and Bachman</td>
<td>Patients with dementia</td>
<td>Not reported</td>
<td>B12 deficiency should be screened for and treated in patients with dementia</td>
</tr>
</tbody>
</table>

\(^{5}\) The Clinical Practice Guideline for anaemia in chronic kidney disease\(^{(77)}\). (KDIGO) KDIGO. KDIGO Clinical Practice Guideline for Anemia in Chronic Kidney Disease2012. is based upon systematic literature searches last conducted in October 2010, supplemented with additional evidence through March 2012.
<table>
<thead>
<tr>
<th>Title</th>
<th>Author and country</th>
<th>Populations</th>
<th>Frequency</th>
<th>Overall recommendation</th>
</tr>
</thead>
</table>
| Chronic fatigue syndrome/myalgic encephalomyelitis (or encephalopathy): diagnosis and management of CFS/ME in adults and children (2007)
| NICE UK                                                            | People with CFS/ME | Not reported                                        | Tests for vitamin B12 deficiency and folate levels should not be carried out unless a full blood count and mean cell volume show a macrocytosis |
| Distal symmetric polyneuropathy                                      | American Academy of Neurology (AAN) | Patients with polyneuropathy                        | Not reported | Serum B12 with metabolites (methylmalonic acid with or without homocysteine) tests may be considered for all patients with polyneuropathy |
| Chohn's disease                                                      | Kulnigg and Gasche Austria | Patients with Crohn’s disease or inflammatory bowel disease | Not reported | Routine vitamin B12 measurements are not necessary, only if patients have macrocytic anaemia or do not respond to iron treatment |

Guidelines from the British Columbia Guidelines and Protocols Advisory Committee (2012) recommend performing a full blood count, blood film and serum cobalamin in all patients suspected of cobalamin deficiency. The guidelines recommend interpreting serum cobalamin levels in light of clinical symptoms, because the test has the following limitations:

- it measures total, not metabolically active cobalamin;
- the levels of cobalamin do not correlate well with clinical symptoms; elderly patients may have normal cobalamin levels with clinically significant cobalamin deficiency, while women taking oral contraceptives may have decreased blood cobalamin levels due to a decrease in transcobalamin, a carrier protein, but no clinical symptoms of deficiency;
- there is a large ‘gray zone’ between the normal and abnormal levels; and
- the reference intervals may vary between laboratories. The guidelines state that the conventional cut-off for serum cobalamin deficiency varies from 150-220 pmol/L.

The best practice review by Smellie et al. (2005) on the diagnosis and monitoring of vitamin B12 (and folate) deficiency was based on a standardised literature search of national and international guidance notes, consensus statements, health policy documents, and evidence based medicine reviews, supplemented by relevant primary research documents. However, the authors of the best practice review stated that the recommendations were mostly
‘consensus rather than evidence based’. Therefore, the guidance/recommendations were derived from a small number of reviews, supplemented by extrapolations from knowledge of the physiology of vitamin B12 and folate. The best practice review recommended that vitamin B12 (and folate) testing should be performed in patients with macrocytic anaemia, macrocytosis, and specific neuropsychiatric abnormalities (including paraesthesia, ataxia, peripheral neuropathy, and memory loss).

The systematic review by Andres et al (2004)\(^{(72)}\) used a flow chart or care pathway to describe recommendations. A notable difference in the guideline by Andres et al (2004) was that the authors recommended screening all patients in institutions or psychiatric hospitals for vitamin B12 deficiency. Andres et al (2004) reported that the prevalence of vitamin B12 deficiency was much higher (30% to 40%) in patients who were sick or institutionalised. As mentioned above for the British Columbia Medical Association guideline, there was a very weak relationship between the recommendations and the evidence presented in all guidelines.

The British Committee for Standards in Haematology (BCSH) published a review entitled "Guidelines on the investigation and diagnosis of cobalamin and folate deficiencies" in 1994\(^{(76)}\). Although now very dated, this publication highlighted that vitamin B12 deficiency was synonymous with macrocytic anaemia, but that many patients with pernicious anaemia may present without either anaemia or macrocytosis.\(^{(76)}\) However, emphasis on haematological indices and blood film examination is strong and the authors did not provide comment on the relative merits of various laboratory and test procedures nor on appropriate testing algorithms.

There are several guidelines that focus on specific clinical conditions, which indicate that serum vitamin B12 levels should be assessed. The clinical conditions include (but not limited to) cognitive impairment\(^{(79, 80)}\), dementia\(^{(81)}\), Crohn’s disease\(^{(84)}\) and chronic fatigue syndrome\(^{(82)}\).

The guideline published by the British Columbia Medical Association and Ministry of Health on cognitive impairment in the elderly stated that data from systematic reviews of RCTs (only one of which was cited, Malouf et al. (2003)\(^{(85)}\)) did not provide evidence of improvement in cognition or dementia with vitamin B12 treatment.

In addition, many guidelines recommended the evaluation of vitamin B12 deficiency in the workup for clinical indications without specifying a methodology. An exception is in a practice parameter for peripheral neuropathy by the American Academy of Neurology (AAN) that has specified a methodology (evidence level C): screening for “serum B12 level with metabolites (methylmalonic acid with or without homocysteine)” in the evaluation for vitamin B12 deficiency in all patients with distal symmetric polyneuropathy.\(^{(83)}\)

### 4.2 Recommendations from other reports

In July 2013, Health Quality Ontario released recommendations on vitamin B12 testing from the Appropriateness Working Group of the Ontario Health Technology Advisory Committee (OHTAC).\(^{(86)}\) The objective of their Appropriateness Initiative is to develop a systematic framework for the ongoing identification, prioritisation, and assessment of health interventions in Ontario for which there is possible misuse, overuse, or underuse. Seven health interventions were examined in the first phase: annual health exams, aspartate aminotransferase testing, ferritin testing, folate testing and vitamin B12 testing. The analysis of vitamin B12 testing was based on a rapid review (see Sections 5.1.2, 5.1.3 and 5.1.6). On
the basis of the findings of the review, OHTAC recommended that vitamin B12 testing is removed from the Ontario laboratory requisition form.\(^{(86)}\)

### 4.3 Summary of clinical guidance

No relevant Australian guidelines were identified in the literature search. Four guidelines on diagnosing vitamin B12 deficiency were identified (two from the UK and two from Canada), with limited evidence supporting the recommendations.

One 2012 guideline from the British Columbia Medical Association and Ministry of Health, Canada, concluded that routine screening for vitamin B12 deficiency is not recommended. No other guidelines made recommendations relating to routine screening.

Several guidelines recommend that patients with symptoms or signs of vitamin B12 deficiency (macrocytic anaemia or macrocytosis) and patients with suspected neuropsychiatric abnormalities should be tested for vitamin B12 deficiency. Other populations where testing could be considered include the elderly, long-term vegans, people on drugs that interfere with vitamin absorption (long-term H2 receptor antagonists or proton pump inhibitors or metformin) and patients with inflammatory bowel disease, gastric or small intestine resection.

The frequency with which patients should be tested was not addressed in the guidelines. However, a 2005 best practice review from the UK stated that there is no obvious merit in repeating vitamin B12 measurements (in patients with macrocytic anaemia, macrocytosis, or patients with specific neuropsychiatric abnormalities) unless lack of compliance is suspected or anaemia recurs.

In July 2013, Health Quality Ontario released recommendations on a variety of common clinical tests, including vitamin B12. On the basis of their rapid review which found that serum vitamin B12 test has low diagnostic accuracy, it was recommended that vitamin B12 testing is removed from the Ontario laboratory requisition form.
5 REVIEW OF THE CLINICAL EVIDENCE FOR VITAMIN B12 TESTING

This Chapter presents the results of the systematic literature review on vitamin B12 testing in relation to the clinical research questions.

5.1 Evidence base

5.1.1 Search results

A literature search was performed on 23rd September 2013, using OVID MEDLINE, EMBASE, and the Cochrane Library, for studies published from January 2006 until September 2013. Abstracts were reviewed and for those studies meeting the eligibility criteria, full-text articles were obtained. Reference lists were also examined for any additional relevant studies not identified through the search. The database search yielded 1,763 citations (with duplicates removed). Articles were excluded based on information in the title and abstract. The full texts of potentially relevant articles were obtained for further assessment.

5.1.2 Existing health technology reports and systematic reviews

The literature search identified a recent Rapid Review on serum vitamin B12 testing(71) published in December 2012 by Health Quality Ontario. The objective of this rapid review was to establish under what circumstances, and how often, serum vitamin B12 tests should be used to assess vitamin B12 deficiency. A systematic literature search was conducted between January 2000 and June 2012 to identify relevant systematic reviews, health technology assessments and meta-analyses. The review’s search was also expanded to include randomised controlled trials (RCTs) and guidelines.

5.2 Appropriate clinical indications for vitamin B12 testing

There were no studies identified that evaluated the clinical indications for vitamin B12 testing. However, several guidelines (described in Chapter 4) recommended the evaluation of vitamin B12 deficiency in the workup for clinical indications (anaemia in CKD, post-gastrectomy patients, elderly with cognitive impairment, neuropathy, as well as patients at risk of vitamin B12 deficiency). In addition, the rapid review by Health Quality Ontario concluded (based on three low-grade guidelines) that patients with symptoms or signs of vitamin B12 deficiency anaemia (macrocytic anaemia) should be tested for vitamin B12 deficiency. However, it stated that it is unclear whether other special populations should be tested for B12 deficiency (e.g. patients with suspect neuropsychiatric abnormalities).(71)

5.3 Evidence that testing for vitamin B12 levels improves health outcomes

No definitive conclusions can be drawn about the effectiveness of vitamin B12 testing since no prospective comparative trials have been conducted to directly assess the impact of testing on health outcomes in healthy populations or in patients with chronic disease associated with vitamin B12 deficiency.

5.4 Risks/harms associated with vitamin B12 testing

No trials designed to directly measure the risks or harms associated with vitamin B12 testing were identified. In terms of harms, vitamin B12 testing relies on a blood draw, which is a safe procedure.
It is likely that the consequences of inaccurate or inappropriately interpreted serum vitamin B12 test results, such as a false positive, are relatively small. Vitamin B12 supplements are generally considered safe when taken in amounts that are not higher than the recommended dietary allowance. Findings from the NORVIT\(^{(87)}\) and HOPE 2\(^{(88)}\) intervention trials support these claims. In both of these trials, vitamin B12 supplementation (in combination with folic acid and vitamin B6) did not cause any serious adverse events when administered at doses of 0.4 mg for 40 months (NORVIT trial) and 1.0 mg for 5 years (HOPE 2 trial).

5.5 Quality of testing according to testing platform

5.5.1 Diagnostic performance of the serum vitamin B12 assay

The Ontario rapid review identified one systematic review and meta-analysis performed by Willis et al (2011)\(^{(89)}\) on the diagnostic accuracy of the serum tests for assessing vitamin B12 (or cobalamin) across patient subgroups. They searched the literature from 1990 to November 2009 and identified 54 studies for inclusion. Of these, 12 were classified as case-control studies, while 42 were classified as cohort studies. The cohort studies were further subdivided into 18 studies enrolling patients with suspected vitamin B12 deficiency (suspicion based on previous investigation, e.g. serum vitamin B12, MMA, homocysteine, clinical signs, etc.), a single study enrolling patients with suspected normal vitamin B12 levels (based on previous investigation, e.g. serum vitamin B12, MMA, homocysteine, clinical signs, etc.) and 23 studies enrolling patients with unknown vitamin B12 status. In this systematic review, the existing evidence base for serum vitamin B12 tests rated poorly against the criteria of a widely used and validated quality assessment tool (QUADAS)\(^6\). Studies that employed a rigorous reference standard method, such as that reported by Matchar et al. (1994)\(^{(90)}\), were rated as positive against the QUADAS criterion relating to reference standards. The absence of a gold standard resulted in many studies being rated as unclear (rather than poor quality) on this criterion.

The included studies compared serum vitamin B12 to a reference standard (all reference standards were employed). They reported that there was no consistent reference standard used to measure the accuracy of the serum vitamin B12 test.\(^7\) They also reported a wide range of variability for sensitivity and specificity across the studies. The review found low levels of test sensitivity and, to a lesser extent, specificity across clinical indications and practice settings. For sensitivity, the range was 13% to 75%, and for specificity the range was 45% to 100%.

Test sensitivity was low when MMA and Hcy were employed as separate reference standards [MMA 0.52 (95% CI: 0.39, 0.65) and Hcy 0.40 (95% CI: 0.27, 0.54)]. Specificity estimates were considerably higher than those estimated for sensitivity [MMA 0.81 (95% CI: 0.70, 0.89) and Hcy 0.84 (95% CI: 0.73, 0.90)]. The authors of the systematic review attributed the wide ranges to the inconsistent use of a reference standard.\(^{(89)}\)

The systematic review by Willis et al. (2011)\(^{(89)}\) included studies that assessed the diagnostic accuracy of serum B12 test using holoTC (four studies) as a reference standard\(^{(51)}\). HoloTC binds only a small portion of the total plasma vitamin B12 (20–30%) but is responsible for delivery of vitamin B12 to cells and is considered to be the functionally active fraction of

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\(^7\) MMA and Hcy are the two most commonly reported reference standards in the literature
vitamin B12\(^{(44)}\) and has been advocated as having comparable diagnostic performance to serum vitamin B12 assays\(^{(46, 51, 52, 91)}\). The meta-analysis performed by Willis et al. (2011) reported that serum B12 test sensitivity was higher when holoTC was used as a referent as compared to when MMA and Hcy were employed as referants (holoTC 0.70, 95% CI: 0.55, 0.82). Specificity was 0.79 (95% CI: 0.68, 0.86) when holoTC was used as the referent.\(^{(89)}\)

Assay methods for serum vitamin B12 analyses varied across studies; however, similar values for sensitivity and specificity were observed for both MMA and Hcy analyses. Radioassays were the most commonly reported methods for quantifying serum vitamin B12 (eight studies for MMA analysis, 11 studies for Hcy analysis), and demonstrated low levels of sensitivity in pooled analyses [MMA 0.57 (95% CI: 0.37, 0.74), Hcy 0.40 (95% CI: 0.24, 0.59)]. Under both reference standards, studies employing chemiluminescence (three studies for MMA analysis, one study for Hcy analysis), which is the most recent technology innovation for quantifying serum vitamin B12, demonstrated significantly lower sensitivity [MMA 0.36 (95% CI: 0.33, 0.38), and Hcy 0.13 (95% CI not reported)] compared to studies using radioimmunoassays (three studies for MMA analysis, two studies for Hcy analysis) [MMA 0.75 (95% CI: 0.66, 0.83), Hcy 0.70 (95% CI: 0.53, 0.83)]. This may indicate that the transition from older assay methods to newer technologies was not associated with improved diagnostic accuracy. However, these results need to be interpreted in light of the limitations of the existing evidence base, particularly the absence of an internationally accepted ‘gold standard’ for diagnosing conditions amenable to vitamin B12 supplementation, existing reference standard imperfections, and evolving meta-analytical methods for pooling data across diagnostic accuracy studies.\(^{(89)}\)

Results of the meta-analysis performed by Willis et al. (2011)\(^{(89)}\) showed that positive likelihood ratios\(^{8}\) (PLR) close to one (suggesting limited value in differentiating between disease presence or absence) were observed for serum vitamin B12 across all subgroups. Among studies employing MMA as the referent, the PLR ranged from 1.23 (95% CI: 0.87, 1.70) in patients with previous vitamin B12 deficiency, to 3.70 (95% CI: 2.66, 5.13). Similar results were found in analyses of Hcy as the referent, with the PLR ranging from 0.90 (95% CI: 0.54, 1.27) to 3.68 (95% CI: 2.77, 4.89) in cohort studies enrolling patients with unknown vitamin B12 status. Negative likelihood ratios\(^{9}\) (NLRs) ranged from 0.95 (95% CI: 0.82, 1.09) in studies restricted to neuropsychiatric patients and Hcy as the reference standard, to 0.34 (95% CI: 0.22, 0.46) for studies using radioimmunoassay and using MMA as the reference standard, or those employing a clinical reference standard [0.34, (95% CI: 0.13, 0.89)]. The overall PLR and NLR were 2.72 (95% CI: 1.95, 3.81) and 0.59 (95% CI: 0.49, 0.72), respectively, in studies employing MMA as the referent. The overall PLR and NLR were 2.40 (95% CI: 1.78, 3.23) and 0.72 (95% CI: 0.62, 0.84), respectively in studies employing Hcy as the referent. In studies employing a clinical reference standard (e.g. response to therapy), PLR was 3.33 (95% CI: 0.92, 12.10) and NLR 0.34 (95% CI: 0.13, 0.89).

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\(^{8}\) The likelihood ratio (LR) of any clinical finding is the probability of that finding in patients with disease divided by the probability of the same finding in patients without disease. Positive likelihood ratio is equivalent to the probability that a person with the disease tested positive for the disease (true positive) divided by the probability that a person without the disease tested positive for the disease (false positive).

\(^{9}\) Negative likelihood ratio (NLR) is equivalent to the probability that a person with the disease tested negative for the disease (false negative) divided by the probability that a person without the disease tested negative for the disease (true positive).
In summary, the 2011 review by Willis et al. was unable to demonstrate that diagnosis of conditions potentially amenable to vitamin B12 supplementation on the basis of a single serum vitamin B12 measurement is an accurate method for identifying vitamin B12 deficient patients. Across clinical indications and practice settings, the authors of the review found low levels of test sensitivity and, to a lesser extent, specificity. While age associated changes in vitamin B12 levels have been reported, the available data did not demonstrate improved sensitivity or specificity of vitamin B12 tests by age group. The transition from older assay methods to newer technologies (e.g. chemiluminescence) was not associated with improved diagnostic accuracy. The authors of the review stated that whilst these results may be suggestive of poor diagnostic accuracy for serum vitamin B12 assay, they need to be interpreted in light of the limitations of the existing evidence base, particularly the absence of an internationally accepted gold standard for diagnosing conditions amenable to vitamin B12 supplementation, existing reference standard imperfections, and evolving meta-analytical methods for pooling data across diagnostic accuracy studies.\(^{(92)}\)

The authors of the review acknowledged that not all variation in serum vitamin B12 test performance seen in the results is attributed to serum vitamin B12 assay deficiency. Therefore, it is wrong to conclude that serum vitamin B12 assays have no role in the diagnostic management of patients with diseases potentially amenable to vitamin B12 supplementation. Attempts have been made to outline diagnostic algorithms using a range of available tests including serum vitamin B12, Hcy and MMA. In combination with individualised patient management, such algorithms may be able to better tailor the use of serum vitamin B12 assays alongside clinical examination. Moreover, the role of serum vitamin B12 in medical decision making and the utility of results to influence therapeutic actions, requires greater clarification to more precisely define the place of this test in the management of patients with potential vitamin B12 deficiency.\(^{(92)}\)

### 5.5.2 Diagnostic performance of the holoTC assay

Numerous other studies (shown in Table 5.1) have compared holoTC’s performance with that of total vitamin B12 for identification of patients with vitamin B12 deficiency.\(^{(46, 51-53, 93-98)}\) In a longitudinal cohort study of 2,403 randomly selected older people, Clarke et al. (2007)\(^{(97)}\) reported slightly superior diagnostic performance of holoTC compared to serum vitamin B12 measures (area under the curve (AUC) 0.85 versus 0.76, p<0.001). Similarly, in a multicentre study of 360 blood samples collected by five Dutch hospitals, Heil et al. (2012)\(^{(53)}\) demonstrated a greater AUC for holoTC than for vitamin B12 in detecting vitamin B12 deficiency characterised by three predefined cut-off levels of MMA (serum MMA >0.32 \(\mu\)mol/L (i.e. 90th percentile); MMA >0.45 \(\mu\)mol/L (i.e. 97.5th percentile); and MMA >0.77 \(\mu\)mol/L (i.e. 99th percentile)). Applying a cut-off value of MMA >0.45 \(\mu\)mol/L resulted in an AUC of 0.70 (95% CI: 0.61, 0.79) for vitamin B12 and AUC of 0.78 (95% CI: 0.69, 0.87) for holoTC (p = 0.06). In addition, a cut-off value of 32 pmol/L of holoTC resulted in the highest sensitivity (83%) with acceptable specificity (60%) in detecting MMA concentrations above 0.45 \(\mu\)mol/L. However, the combination of vitamin B12 and holoTC in this study did not improve the diagnostic accuracy at this cut-off level. A third study by Herrmann and Obeid (2013)\(^{(96)}\) also evaluated the diagnostic accuracy of holoTC assay compared to serum vitamin B12 on 1,359 serum samples. The authors of this study demonstrated that holoTC showed a higher AUC curve compared to serum vitamin B12 for detecting MMA levels > 300 nM. The AUC results of the other studies are summarised in Table 5.1.
Table 5.1: Comparison of holotranscobalamin (holoTC) and total serum vitamin B12 for diagnosis of vitamin B12 deficiency

<table>
<thead>
<tr>
<th>Author</th>
<th>Total number of subjects (total number of subjects with vitamin B12 deficiency)</th>
<th>Age (years)</th>
<th>Limits for MMA (tHcy) (µmol/L)</th>
<th>AUC for holoTC</th>
<th>AUC for vitamin B12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herrmann et al. (2003)</td>
<td>204 (68)</td>
<td>21-73</td>
<td>&gt;0.27</td>
<td>0.88</td>
<td>0.84</td>
</tr>
<tr>
<td>Lloyd-Wright et al. (2003)</td>
<td>172 (36)</td>
<td>&gt;18</td>
<td>&gt;0.75</td>
<td>0.87</td>
<td>0.86</td>
</tr>
<tr>
<td>Hvas and Nexo (2005)</td>
<td>806 (24)</td>
<td>&gt;18</td>
<td>&gt;0.75</td>
<td>0.90</td>
<td>0.85</td>
</tr>
<tr>
<td>Miller et al. (2006)</td>
<td>1,789 (116)</td>
<td>≥60</td>
<td>&gt;0.35</td>
<td>0.83</td>
<td>0.82</td>
</tr>
<tr>
<td>Clarke et al. (2007)</td>
<td>2,403 (129)</td>
<td>&gt;65</td>
<td>&gt;0.75</td>
<td>0.85</td>
<td>0.76</td>
</tr>
<tr>
<td>Obeid and Herrmann (2007)</td>
<td>759 (174)</td>
<td>8-92</td>
<td>&gt;0.30</td>
<td>0.71</td>
<td>0.60</td>
</tr>
<tr>
<td>Schrempf et al. (2011)</td>
<td>1,279 (71)</td>
<td>18-98</td>
<td>&gt;0.40</td>
<td>0.66</td>
<td>0.72</td>
</tr>
<tr>
<td>Valente et al. (2011)</td>
<td>700 (not reported)</td>
<td>63-97</td>
<td>&gt;0.36</td>
<td>0.90</td>
<td>0.80</td>
</tr>
<tr>
<td>Heil et al. (2012)</td>
<td>360†(47)</td>
<td>≥18</td>
<td>&gt;0.45</td>
<td>0.78</td>
<td>0.70</td>
</tr>
</tbody>
</table>

† Blood samples

All but one of the studies in Table 5.1 showed that holoTC’s performance is better than that of vitamin B12, or comparable to serum vitamin B12, independent of the cut-off MMA value they used to classify patients’ vitamin B12 status. The study by Schrempf et al. (2011) was the only study that reported that the holoTC assay did not show superior diagnostic accuracy compared to total serum vitamin B12 assay for the detection of vitamin B12 deficiency in a cohort of subjects with neuropsychiatric conditions. The AUCs were not significantly different for holoTC compared to vitamin B12 in all subjects (AUC: 0.66 [95% CI: 0.51, 0.82]; p = 0.04 vs. 0.72 [95% CI: 0.65, 0.78], p < 0.0001) (Table 5.1).

However, the primary limitation of most of these studies is that comprehensive clinical diagnostic criteria were not used to definitively categorise individuals as vitamin B12 deficient or vitamin B12 adequate. The authors of these studies acknowledge that they had no access to haematologic or neurologic assessments. Consequently, they could only categorise persons as having likely vitamin B12 deficiency based on elevated MMA and homocysteine concentrations, while accounting for potential confounding by renal dysfunction (all the studies shown in Table 5.1 involved patients with normal kidney function).

Another limitation is that ‘falsely’ elevated holoTC has been noted in patients with common genetic polymorphisms, impaired renal function and liver disease. Like other biological markers of vitamin B12 status (MMA and Hcy), the influence of factors other than vitamin B12 levels on holoTC status has considerable effects on its utility, not only as a diagnostic tool but as a useful reference standard for evaluating serum vitamin B12 performance.
In summary, there is evidence which indicates that holoTC has a comparable or better diagnostic accuracy to that of total serum vitamin B12. There is insufficient evidence to establish holoTC testing as an alternative to either total serum vitamin B12 or levels of MMA or homocysteine in the diagnosis of vitamin B12 deficiency.
6 REVIEW OF THE ECONOMIC EVIDENCE FOR VITAMIN B12 TESTING

This Chapter presents a preliminary economic evaluation of vitamin B12, which is limited to a summary of the findings from studies identified through the systematic literature review. A formal modelled economic evaluation of vitamin B12 testing was not within the scope of this review.

6.1 Studies relevant to the economic evaluation of vitamin B12 testing

No relevant studies were identified.
7 FINDINGS AND CONCLUSIONS

This Chapter sets out the findings and conclusions of the review of vitamin B12 testing – as represented by MBS item numbers 66599 and 66602 – based on the analysis of the available MBS data; evidence obtained through systematic literature review; and the information derived from the stakeholder consultations.

7.1 Current usage of vitamin B12 and/or folate testing services in Australia

Over the past 10 years, the number of claims for MBS item 66599 has more than doubled (+119%) from 282,531 in 2003/04 to 618,744 in 2012/13. Over the same timeframe the number of claims for MBS item 66602 has had an even greater increase (+307%) from 522,980 to 2,129,051. The increase in benefits paid for both items reflects the increase in claims. Benefits paid for MBS item 66599 increased from $5.7m to $12.5m (+120%), whereas benefits for MBS item 66602 increased from $19.2m to $78.5m (+309%). While total benefits increased significantly, the proportion of benefits paid to each state and territory remained relatively constant over the ten-year period. The highest proportion of benefits paid over the past ten years was in New South Wales (34% of total for 66599 and 38% for 66602), followed by Victoria (30% of total for 66599 and 28% for 66602).

To further explore geographical trends in testing, an analysis was conducted of the number of services per capita (i.e. per 100,000 population), according to the address at the time of claiming of the patient to whom the service was rendered. In 2012/13, there were 2,666 claims per 100,000 people enrolled in Medicare across Australia for item 66599 and 9,172 claims per 100,000 people for item 66602. South Australia had the highest rate of claiming for item 66599 per capita (3,635 claims per 100,000 population), while the lowest per capita rate was in the Northern Territory (762 claims per 100,000 population). For item 66602, the highest number of claims per capita in 2012/13 was for NSW and Victoria (over 10,000 claims per 100,000 population in both states), while Tasmania had the lowest (less than 4,000 claims per 100,000 population).

MBS item numbers 66599 and 66602 are claimed by both males and females; however, females had a higher number of tests at all ages, except in the youngest age category (< 5 years). Females also had a steeper increase in testing volume than males, with the largest difference between genders in the 15-24 and 25-34 year age groups. For item 66599, the number of tests being performed in people aged 45 years and over was 76% for males and 64% for females. For item 66602, 71% of claims for males were aged 45 years and over versus 60% for females. For females, testing decreased from age 65 years. Similarly, the number of tests dropped dramatically in elderly men.

For both MBS items, there was an increase in the overall number of patients tested between 2008/09 and 2012/13. However, there was very little change in the proportion of patients receiving either one test per year (92% and 90% for items 66599 and 66602, respectively), two tests per year (7.5% and 9% for items 66599 and 66602, respectively), or three or more tests per year (0.8% for item 66599 and 1.1% for item 66602). These data suggest that the majority of vitamin B12/folate testing services are being undertaken for the purposes of screening/testing rather than monitoring.
Over the five-year time period from 2008/09 to 2012/13, there were no material changes in the pattern of requesting providers. General practitioners and other medical practitioners accounted for approximately 71% and 67% of all providers requesting item 66599 and item 66602, respectively. Approximately 14% of providers requesting vitamin B12/folate testing were internal medicine consultant physicians. There was a large variety of other providers requesting services, but they each accounted for less than 4% of provider counts.

For both items, there was an increase over the period 2008/09 to 2012/13 in the overall number of providers requesting vitamin B12/folate testing. For item 66599, the majority of providers (97%) requested 100 or fewer tests per year. Approximately 2% of providers requested 101 to 200 tests, and the remaining providers requested more than 200 tests per year. There were a small number of providers (ranging from 36 to 62 per year) that requested more than 400 tests per year. Approximately 88% of the providers requesting item 66602 requested 100 or fewer tests per year. Approximately 7% of providers requested between 101 and 200 tests per year and 2.5% requested 201 to 300 tests per year. Between 2010/11 and 2012/13, more than 500 providers requested over 400 tests per year.

7.2 Clinical guidance on vitamin B12 testing

The MBS data indicates that the majority of requests for vitamin B12 testing are initiated by GPs and OMPs. However, there were no Australian guidelines identified in the literature search that provide practice advice on vitamin B12 testing. Several international guidelines relating to the use of vitamin B12 testing vary widely in their recommendations. While some recommend vitamin B12 testing as a screening tool in commonly encountered illnesses such as dementia, others suggest restricting testing to patients who have already undergone pre-test investigations (such as full blood examinations and blood film examination). There were four international guidelines that were identified on the diagnosis of vitamin B12 deficiency. The 2012 Canadian guideline by the British Columbia Medical Association and Ministry of Health does not recommend routine screening of vitamin B12 deficiency. An old (1994) guideline by the British Committee for Standards in Haematology recommended that serum B12 testing is carried out following an initial investigation (full blood count and blood film examination). A Canadian review by Andres et al. (2004) recommended that all people over the age of 65 years who are malnourished, all people in institutions or psychiatric hospitals, and all people with haematological or neuropsychiatric manifestations of vitamin B12 deficiency should have their serum vitamin B12 levels measured. Smellie and colleagues (2005) recommended that vitamin B12 testing should be conducted following proper assessment of the presence of deficiency through collection of accurate information on a patient’s medical history, medication, alcohol intake and symptoms of malabsorption. The identified guidelines on diagnosing vitamin B12 deficiency have limited evidence supporting the recommendations.

Almost all guidelines did not advise on the frequency of testing. The review by Smellie et al. (2005) advised against the repeated testing of vitamin B12 unless there is a lack of patient response to treatment or if anaemia reoccurs. This is consistent with the lack of direct evidence that vitamin B12 testing improves health outcomes. There is no moderate to high level evidence pertaining to the clinical utility of vitamin B12 testing.

Only the practice parameter, published by the American Academy of Neurology, specified a methodology for vitamin B12 measurement. This advised using serum vitamin B12 level with metabolites (MMA and Hcy) in the evaluation of vitamin B12 deficiency in all patients with distal symmetric neuropathy. None of the other guidelines described in this report specify a methodology for vitamin B12 measurement.
None of the guidelines advised on the diagnostic accuracy of the different tests used to assess vitamin B12 deficiency or advised on which metabolite is the best indicator of vitamin B12 status. This may be attributed to the lack of a gold reference standard for vitamin B12.

7.3 Relationship between testing for vitamin B12 levels and health outcomes

No definitive conclusions can be drawn about the effectiveness of vitamin B12 testing since no prospective comparative trials have been conducted to directly assess the impact of testing on health outcomes in healthy populations or in patients with chronic disease associated with vitamin B12 deficiency.

7.4 Harms associated with vitamin B12 testing

No trials designed to directly measure the risks or harms associated with vitamin B12 testing were identified. However, vitamin B12 testing relies on a blood draw, which is a safe procedure. Vitamin B12 supplements are generally considered safe when taken in amounts that are not higher than the recommended dietary allowance. Thus, it is likely that the consequences of inaccurate or inappropriately interpreted serum vitamin B12 test results, such as a false positive, are relatively small.

7.5 Diagnostic performance of vitamin B12 tests

A 2011 systematic review by Willis and colleagues evaluated the diagnostic accuracy of serum B12 tests using MMA, Hcy and holoTC as a reference standard. The review noted that the available evidence on the diagnostic accuracy of the serum vitamin B12 test is low, due to the lack of a gold reference standard. From the available evidence, diagnosis of conditions amenable to vitamin B12 supplementation on the basis of serum vitamin B12 levels alone cannot be considered a reliable approach to investigating suspected vitamin B12 deficiency. Across clinical indications, practice settings and different methodologies, the authors of the review found low levels of test sensitivity and, to a lesser extent, specificity. The authors of the review also demonstrated that the transition from older assay methods to newer technologies, such as chemiluminescence, was not associated with improved diagnostic accuracy.

Important findings from the 2011 review are summarised below:

- There is currently no consensus on the best method to estimate vitamin B12 deficiency and vitamin B12 status.
- Measurement of total serum vitamin B12 is widely used as a standard screening test but there are problems with the sensitivity and specificity of this test.
- The determination of serum vitamin B12 diagnostic accuracy is difficult to establish due to the absence of a gold standard.
- The accuracy of the serum vitamin B12 assay differs according to existing reference standards (MMA, Hcy, or holoTC) and subgroups; however, not all variation in serum vitamin B12 test performance may be attributed to serum vitamin B12 assay deficiency.
- The absence of a gold standard method hampers the ability to compare the various markers of B12 deficiency (MMA, Hcy, and holoTC) with each other.
- All four biochemical tests have poor specificity.
There is evidence which indicates that holoTC has a comparable or better diagnostic accuracy to that of total serum vitamin B12. This was demonstrated in nine comparative studies that reported a significantly greater area under the curve for holoTC than for vitamin B12 in detecting vitamin B12 deficiency. Only one study reported that the holoTC assay has poorer diagnostic accuracy than the serum vitamin B12 assay. However, there is insufficient evidence to establish holoTC testing as an alternative to either total serum vitamin B12, or levels of MMA or homocysteine in the diagnosis of vitamin B12 deficiency.

7.6 Cost implications of vitamin B12 testing

It is unknown whether measurement of vitamin B12 levels is cost-effective. No costing studies or economic analyses of vitamin B12 testing were identified.

7.7 Conclusions

There has been a substantial increase in the number of claims for vitamin B12/folate testing over the past ten years. Analysis of MBS data indicates that the majority of vitamin B12 testing services are requested by GPs and OMPs for the purposes of screening or testing, rather than follow-up monitoring. There are no Australian clinical practice guidelines that either advocate or recommend against routine testing for vitamin B12. The international clinical practice guidelines vary widely in their recommendations. While some recommend vitamin B12 test as screening tools in commonly encountered illnesses such as dementia, others suggest restricting testing to patients who have already undergone pre-test investigations such as a full blood count or blood film examination. There are no recommendations on the frequency of vitamin B12 testing and there is no direct evidence regarding the clinical utility of vitamin B12 testing in any population.
APPENDIX 1 – References


77. (KDIGO) KDIGO. KDIGO Clinical Practice Guideline for Anemia in Chronic Kidney Disease2012.


APPENDIX 2 – Review Consultation Committee members

As part of the MBS Review process, the Department established a Review Consultation Committee (RCC). The RCC is a time-limited committee of nominated representatives to provide advice to the Department to inform the review process, such as the development of review reports, i.e. scope and protocol documents, clinical practice and policy issues.

<table>
<thead>
<tr>
<th>Name</th>
<th>Representing</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/Prof Ken Sikaris</td>
<td>Royal College of Pathologists of Australasia</td>
</tr>
<tr>
<td>A/Prof Hans Schneider</td>
<td>Royal College of Pathologists of Australasia</td>
</tr>
<tr>
<td>Dr Zhong Lu</td>
<td>Royal College of Pathologists of Australasia</td>
</tr>
<tr>
<td>Dr Paul Glendenning</td>
<td>Royal College of Pathologists of Australasia</td>
</tr>
<tr>
<td>Dr Richard Whiting</td>
<td>Australian Medical Association</td>
</tr>
<tr>
<td>Dr Andrew Boyd</td>
<td>NPS Medicinewise</td>
</tr>
<tr>
<td>Dr Ie-Wen Sim</td>
<td>Endocrine Society of Australia</td>
</tr>
<tr>
<td>Dr Peter Harman</td>
<td>In-vitro Diagnostic (IVD) Australia</td>
</tr>
<tr>
<td>Dr Dan McLaughlin</td>
<td>Australian and New Zealand Association of Neurologists</td>
</tr>
<tr>
<td>Dr Walid Jammal</td>
<td>General Practitioner</td>
</tr>
<tr>
<td>Chair and Secretariat</td>
<td>MSAC Evaluation Sub-Committee (ESC) member</td>
</tr>
<tr>
<td></td>
<td>Department of Health</td>
</tr>
</tbody>
</table>
APPENDIX 3 – MBS information

The MBS item numbers for vitamin B12 testing in scope of this review include 66599 and 66602 (see Table A3.1). Both of the items relate to testing serum vitamin B12 or testing red cell (or serum) folate. Both of the items are subject to Rule 21 (i.e. no more than three of any combination of these tests are eligible for Medicare subsidy per patient per year).

Table A3.1: Description of folate testing funded under the MBS

<table>
<thead>
<tr>
<th>Item number</th>
<th>MBS item number description</th>
<th>Fee:</th>
<th>Benefit:</th>
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</thead>
<tbody>
<tr>
<td>66599</td>
<td>Serum B12 or red cell folate and, if required, serum folate (Item is subject to Rule 21)</td>
<td>$23.60</td>
<td>75% = $17.70, 85% = $20.10</td>
</tr>
<tr>
<td>66602</td>
<td>Serum B12 and red cell folate and, if required, serum folate (Item is subject to Rule 21)</td>
<td>$42.95</td>
<td>75% = $32.25, 85% = $36.55</td>
</tr>
</tbody>
</table>

Both of the items are subject to Rule 21:

**Serum B12 and red cell folate testing**

21.(1) For items 66599 and 66602, a medicare benefit is not payable for more than 3 episodes of services described in item 66599 or 66602, or any combination of those items, in a 12 month period.

21.(2) A medicare benefit is not payable for a service described in item 66599 if the service was provided as part of the same patient episode as a service described in item 66602.

Source: Department of Human Services – Medicare Australia, accessed September 2013

Table A3.2 shows when the in-scope MBS item numbers were included on the MBS.

Table A3.2: Item number, descriptor and schedule fee start dates for MBS item numbers

<table>
<thead>
<tr>
<th>MBS item number</th>
<th>Type of date</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>66599</td>
<td>Item Start Date</td>
<td>01-Nov-1998</td>
</tr>
<tr>
<td></td>
<td>Current Descriptor Start Date</td>
<td>01-Mar-1999</td>
</tr>
<tr>
<td></td>
<td>Current Schedule fee start date</td>
<td>01-Jan-2013</td>
</tr>
<tr>
<td>66602</td>
<td>Item Start Date</td>
<td>01 Nov 1998</td>
</tr>
<tr>
<td></td>
<td>Current Descriptor Start Date</td>
<td>01 Mar 1999</td>
</tr>
<tr>
<td></td>
<td>Current Schedule fee start date</td>
<td>01-Jan-2013</td>
</tr>
</tbody>
</table>

Source: Department of Human Services – Medicare Australia, accessed September 2013
APPENDIX 4 – Search term strategy

The literature search strategies focused on the clinical evidence for vitamin B12 testing (Table A4.1) and the cost implications associated with vitamin B12 testing (Table A4.2).

Table A4.1: Search strategy for clinical evidence

<table>
<thead>
<tr>
<th>Population</th>
<th>Embase and Medline</th>
<th>Search Terms</th>
</tr>
</thead>
<tbody>
<tr>
<td>General healthy population</td>
<td>Population – (‘pregnancy’/exp OR ‘pregnancy’) OR (‘infant’/exp OR ‘infant’) OR (‘human milk’/exp OR ‘human milk’) OR (‘lactation’/exp OR ‘lactation’) OR (‘vegetarian’/exp OR ‘vegetarian’) OR (‘malnutrition’/exp OR ‘malnutrition’) OR (‘elderly’/exp OR ‘elderly’) OR (‘aged’/exp OR ‘aged’) OR (‘gluten free diet’/exp OR ‘gluten free diet’) OR (‘alcoholism’/exp OR ‘alcoholism’)) AND Intervention – (‘vit* B12’ OR ‘vitamin B12’/exp OR ‘vitamin B12’ OR cobalamin OR cyanocobalamin OR hydroxycobalamin OR methylcobalamin OR ‘methylmalonic acid’/exp OR ‘methylmalonic acid’/exp OR ‘methylmalonic acid’ OR ‘MMA’ OR ‘methylmalonate’ OR ‘malonic acid’ OR ‘holotranscobalamin’/exp OR ‘holotranscobalamin’ OR ‘holoTC’/exp OR ‘holoTC’) AND (‘testing’/exp OR ‘testing’ OR ‘haematologic test*’/exp OR ‘haematologic test*’) AND Limits – [humans]/lim AND [english]/lim</td>
<td></td>
</tr>
<tr>
<td>Cochrane Library</td>
<td>Population – ((MeSH descriptor Pregnancy explode all trees) OR (MeSH descriptor Infant explode all trees) OR (MeSH descriptor Human Milk explode all trees) OR (MeSH descriptor Lactation explode all trees) OR (MeSH descriptor Vegetarian explode all trees) OR (MeSH descriptor Malnutrition explode all trees) OR (MeSH descriptor Aged explode all trees) OR (MeSH descriptor Alcoholism explode all trees) OR ((pregnancy) OR (pregnancy):ti,ab,kw) OR ((infant) OR (infant):ti,ab,kw) OR ((human milk) OR (human milk):ti,ab,kw) OR ((lactation) OR (lactation):ti,ab,kw) OR ((vegetarian) OR (vegetarian):ti,ab,kw) OR ((malnutrition) OR (malnutrition):ti,ab,kw) OR ((aged) OR (aged):ti,ab,kw) OR ((gluten free diet) OR (gluten free diet):ti,ab,kw) OR ((alcoholism) OR (alcoholism):ti,ab,kw)</td>
<td></td>
</tr>
<tr>
<td>Patients diagnosed with anaemia</td>
<td>Embase and Medline</td>
<td>Population – (‘anaemia’/exp OR ‘anaemia’ OR ‘anemia’/exp OR ‘anemia’) OR (‘macrocyt*’/exp OR ‘macrocyt*’) OR (‘megaloblastic’/exp OR ‘megaloblastic’) OR (‘pernicious’/exp OR ‘pernicious’) OR (‘pancytopenia’/exp OR</td>
</tr>
</tbody>
</table>
Population: 'pancytopenia') AND NOT ('iron deficiency anaemia'/exp OR 'iron deficiency anaemia')
AND
Intervention: (Vit*B12 OR ‘vitamin B12'/exp OR ‘vitamin B12’ OR cobalamin OR cyanocobalamin OR hydroxycobalamin OR methylcobalamin OR ‘methylmalonic acid'/exp OR 'methylmalonic acid'/exp OR ‘methylmalonic acid’ OR ‘MMA' OR ‘methylmalonate’ OR ‘malonic acid’ OR ‘holotranscobalamin'/exp OR ‘holotranscobalamin’ OR 'holoTC'/exp OR ‘holoTC') AND ('testing'/exp OR ‘testing’ OR ‘haematologic test*'/*exp OR ‘haematologic test*')
AND
Limits: [humans]/lim AND [english]/lim

Cochrane Library
Population: ((MeSH descriptor Anaemia explode all trees) OR (MeSH descriptor Megaloblastic explode all trees) OR (MeSH descriptor Pernicious explode all trees) OR (MeSH descriptor Pancytopenia explode all trees) OR ((anaemia) OR (anaemia):ti,ab,kw) OR ((megaloblastic) OR (megaloblastic):ti,ab,kw) OR (macrocyt*) OR ((pernicious) OR (pernicious):ti,ab,kw) OR ((pancytopenia):ti,ab,kw ) AND NOT ((MeSH descriptor Iron deficiency anaemia) OR (iron deficiency anaemia):ti,ab,kw)
AND
Intervention: ((MeSH descriptor Vitamin B12 explode all trees) OR (Vitamin B12):ti,ab,kw OR (MeSH descriptor Cobalamin explode all trees) OR (cobalamin):ti,ab,kw OR (MeSH descriptor Cyanocobalamin explode all trees) OR (cyanocobalamin):ti,ab,kw OR (MeSH descriptor Hydroxycobalamin explode all trees) OR (hydroxycobalamin):ti,ab,kw OR (MeSH descriptor Methylcobalamin explode all trees) OR (methylcobalamin):ti,ab,kw OR (MeSH descriptor Methylmalonic acid explode all trees) OR (methylmalonic acid):ti,ab,kw OR (MeSH descriptor Holotranscobalamin explode all trees) OR (holotranscobalamin):ti,ab,kw OR (MeSH descriptor Holotranscobalamin explode all trees) OR (holotranscobalamin):ti,ab,kw OR (MeSH descriptor Holotranscobalamin explode all trees) OR (holotranscobalamin):ti,ab,kw OR (MeSH descriptor Holotranscobalamin explode all trees) OR (holotranscobalamin):ti,ab,kw OR (MeSH descriptor Holotranscobalamin explode all trees) OR (holotranscobalamin):ti,ab,kw)
AND
Limits: [humans]/lim AND [english]/lim

Patients with neurologic disease

Embase and Medline
Population: ('paresthesias'/exp OR 'paresthesias') OR ('peripheral neuropathy'/exp OR 'peripheral neuropathy') OR ('combined system disease'/exp OR 'combined systems disease')
AND
Intervention: (Vit*B12 OR ‘vitamin B12'/exp OR ‘vitamin B12’ OR cobalamin OR cyanocobalamin OR hydroxycobalamin OR methylcobalamin OR ‘methylmalonic acid'/exp OR 'methylmalonic acid'/exp OR ‘methylmalonic acid’ OR ‘MMA' OR ‘methylmalonate’ OR ‘malonic acid’ OR ‘holotranscobalamin'/exp OR ‘holotranscobalamin’ OR 'holoTC'/exp OR ‘holoTC') AND ('testing'/exp OR ‘testing’ OR ‘haematologic test*'/*exp OR ‘haematologic test*')
AND
Limits: [humans]/lim AND [english]/lim

Cochrane Library
Population: ((MeSH descriptor Paresthesias explode all trees) OR (MeSH descriptor Peripheral Neuropathy explode all trees) OR (MeSH descriptor Combined Systems Disease explode all trees) OR ((paresthesias) OR (paresthesias):ti,ab,kw) OR ((peripheral neuropathy) OR (peripheral neuropathy):ti,ab,kw) OR ((combined systems disease) OR (combined systems disease)
<table>
<thead>
<tr>
<th>Population</th>
<th>Search Terms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with gastrointestinal and malabsoption diseases</td>
<td>Embase and Medline Population – ((‘atrophic body gastritis’/exp OR ‘atrophic body gastritis’) OR (‘gastrectomy’/exp OR ‘gastrectomy’) OR (‘gastric sleeve’/exp OR ‘gastric sleeve’) OR (‘peptic ulcer’/exp OR ‘peptic ulcer’) OR (‘H. Pylori’/exp OR ‘H. Pylori’) OR (‘dyspepsia’/exp OR ‘dyspepsia’) OR (‘diarrhoea’/exp OR ‘diarrhoea’) OR (‘coeliac disease’/exp OR ‘coeliac disease’) OR (‘Crohn’s disease’/exp OR ‘Crohn’s disease’) OR (‘tapeworms’/exp OR ‘tapeworms’)) AND Intervention – (Vit<em>B12 OR ‘vitamin B12’/exp OR ‘vitamin B12’ OR cobalamin OR cyanocobalamin OR hydroxycobalamin OR methylcobalamin OR ‘methylmalonic acid’/exp OR ‘methylmalonic acid’/exp OR ‘methylmalonic acid’ OR ‘MMA’ OR ‘methylmalonate’ OR ‘malonic acid’ OR ‘holotranscobalamin’/exp OR ‘holotranscobalamin’ OR ‘holoTC’/exp OR ‘holoTC’) AND (‘testing’/exp OR ‘testing’ OR ‘haematologic test</em>’/exp OR ‘haematologic test*’) AND Limits – [humans]/lim AND [english]/lim</td>
</tr>
</tbody>
</table>
| Cochrane Library Population – ((MeSH descriptor Atrophic Body Gastritis explode all trees) OR (MeSH descriptor Gastrectomy explode all trees) OR (MeSH descriptor Gastric Sleeve explode all trees) OR (MeSH descriptor Peptic Ulcer explode all trees) OR (MeSH descriptor H. pylori explode all trees) OR (MeSH descriptor Dyspepsia explode all trees) OR (MeSH descriptor Diarrhoea explode all trees) OR (MeSH descriptor Coeliac Disease explode all trees) OR (MeSH descriptor Crohn’s Disease explode all trees) OR (MeSH descriptor Tapeworms explode all trees) OR (atrophic body gastritis) OR (atrophic body gastritis):ti,ab,kw OR (gastrectomy) OR (gastrectomy):ti,ab,kw OR (gastric sleeve) OR (gastric sleeve):ti,ab,kw OR (peptic ulcer) OR (peptic ulcer):ti,ab,kw OR (h. pylori) OR (h. pylori):ti,ab,kw OR (dyspepsia) OR (dyspepsia):ti,ab,kw OR (diarrhoea) OR (diarrhoea):ti,ab,kw OR (coeliac disease) OR (coeliac disease):ti,ab,kw OR (Crohn’s disease) OR (Crohn’s disease):ti,ab,kw OR (tapeworms) OR (tapeworms):ti,ab,kw ) AND Intervention – ((MeSH descriptor Vitamin B12 explode all trees) OR (Vitamin B12):ti,ab,kw OR (MeSH descriptor Cobalamin explode all trees) OR (cobalamin):ti,ab,kw OR (MeSH descriptor Cyanocobalamin explode all trees) OR (cyanocobalamin):ti,ab,kw OR (MeSH descriptor Hydroxycobalamin explode all trees) OR (hydroxycobalamin):ti,ab,kw OR (MeSH descriptor Methylcobalamin explode all trees) OR (methylcobalamin):ti,ab,kw OR (MeSH descriptor Methylmalonic acid explode all trees) OR (methylmalonic acid):ti,ab,kw)
<table>
<thead>
<tr>
<th>Population</th>
<th>Search Terms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embase and Medline</td>
<td>Population – (‘dementia’/exp OR ‘dementia’) OR (‘depression’/exp OR ‘depression’) OR (‘psychosis’/exp OR ‘psychosis’) OR (‘Alzheimer’s disease’/exp OR ‘Alzheimer’s disease’)) AND Intervention – (Vit<em>B12 OR ‘vitamin B12’/exp OR ‘vitamin B12’ OR cobalamin OR cyanocobalamin OR hydroxycobalamin OR methylcobalamin OR ‘methylmalonic acid’/exp OR ‘methylmalonic acid’/exp OR ‘methylmalonic acid’) OR ‘MMA’ OR ‘methylmalonate’ OR ‘malonic acid’ OR ‘holotranscobalamin’/exp OR ‘holotranscobalamin’ OR ‘holoTC’/exp OR ‘holoTC’) AND (‘testing’/exp OR ‘testing’ OR ‘haematologic test</em>’/exp OR ‘haematologic test*’) AND Limits – [humans]/lim AND [english]/lim</td>
</tr>
<tr>
<td>Cochrane Library</td>
<td>Population – ((MeSH descriptor Dementia explode all trees) OR (MeSH descriptor Depression explode all trees) OR (MeSH descriptor Psychosis explode all trees) OR (MeSH descriptor Alzheimer’s disease explode all trees) OR ((dementia) OR (dementia):ti,ab,kw) OR ((depression) OR (depression):ti,ab,kw) OR ((psychosis) OR (psychosis):ti,ab,kw) OR ((Alzheimer’s disease) OR (Alzheimer’s disease):ti,ab,kw)) AND Intervention – ((MeSH descriptor Vitamin B12 explode all trees) OR (Vitamin B12):ti,ab,kw OR (MeSH descriptor Cobalamin explode all trees) OR (cobalamin):ti,ab,kw OR (MeSH descriptor Cyanocobalamin explode all trees) OR (cyanocobalamin):ti,ab,kw OR (MeSH descriptor Hydroxycobalamin explode all trees) OR (hydroxycobalamin):ti,ab,kw OR (MeSH descriptor Methylcobalamin explode all trees) OR (methylcobalamin):ti,ab,kw OR (MeSH descriptor Methylmalonic acid explode all trees) OR (methylmalonic acid):ti,ab,kw OR (MeSH descriptor Methylmalonate explode all trees) OR (methylmalonate):ti,ab,kw OR (MeSH descriptor Malonic acid explode all trees) OR (malonic acid):ti,ab,kw OR (MeSH descriptor Holotranscobalamin explode all trees) OR (holotranscobalamin):ti,ab,kw OR (MeSH descriptor HoloTC explode all trees) OR (holoTC):ti,ab,kw ) ) AND ((MeSH descriptor Testing explode all trees) OR (Testing):ti,ab,kw OR (MeSH descriptor Haematologic test* explode all trees) OR (Haematologic test*):ti,ab,kw) AND Limits [humans]/lim AND [english]/lim</td>
</tr>
</tbody>
</table>
### Table A4.2: Search strategy for economic evidence

<table>
<thead>
<tr>
<th>Population</th>
<th>Search Terms</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Population</strong></td>
<td><strong>Search Terms</strong></td>
</tr>
<tr>
<td>Patients undertaking vitamin B12 testing</td>
<td><em>Embase and Medline</em></td>
</tr>
<tr>
<td></td>
<td>Intervention – (Vit*B12 OR ‘vitamin B12’/exp OR ‘vitamin B12’ OR cobalamin OR cyanocobalamin OR</td>
</tr>
<tr>
<td></td>
<td>hydroxycobalamin OR methylcobalamin OR ‘methylmalonic acid’/exp OR ‘methylmalonic acid’/exp OR</td>
</tr>
<tr>
<td></td>
<td>‘methylmalonic acid’ OR ‘methylmalonate’ OR ‘methylmalonate’ OR ‘malonic acid’ OR ‘holotranscobalamin’/exp OR</td>
</tr>
<tr>
<td></td>
<td>‘holotranscobalamin’ OR ‘holoTC’/exp OR ‘holoTC’) AND (‘testing’/exp OR ‘testing’ OR ‘haematologic test’)/exp OR</td>
</tr>
<tr>
<td></td>
<td>‘haematologic test’/)</td>
</tr>
<tr>
<td></td>
<td><strong>Economic Terms</strong> – (‘economic aspect’/exp OR ‘cost benefit analysis’ OR cost* OR ‘cost effectiveness’)</td>
</tr>
<tr>
<td></td>
<td><strong>AND</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Limits</strong> – [humans]/lim AND [english]/lim</td>
</tr>
<tr>
<td></td>
<td><strong>Cochrane Library</strong></td>
</tr>
<tr>
<td></td>
<td>Intervention – ((MeSH descriptor Vitamin B12 explode all trees) OR (Vitamin B12):ti,ab,kw OR (MeSH descriptor Cobalamin explode all trees) OR (cobalamin):ti,ab,kw OR (MeSH descriptor Cyanocobalamin explode all trees) OR (cyanocobalamin):ti,ab,kw OR (MeSH descriptor Hydroxycobalamin explode all trees) OR (hydroxycobalamin):ti,ab,kw OR (MeSH descriptor Methylcobalamin explode all trees) OR (methylcobalamin):ti,ab,kw OR (MeSH descriptor Methylmalonic acid explode all trees) OR (methylmalonic acid):ti,ab,kw OR (MeSH descriptor Methylmalonate explode all trees) OR (methylmalonate):ti,ab,kw OR (MeSH descriptor Malonic acid explode all trees) OR (malonic acid):ti,ab,kw OR (MeSH descriptor Holotranscobalamin explode all trees) OR (holotranscobalamin):ti,ab,kw OR (MeSH descriptor Holotranscobalamin explode all trees) OR (holoTC):ti,ab,kw ) AND ((MeSH descriptor Testing explode all trees) OR (Testing):ti,ab,kw OR (MeSH descriptor Haematologic test explode al trees) OR (Haematologic test*):ti,ab,kw) AND <strong>Economic Terms</strong> – (((economic aspect) OR (economic aspect):kw) OR (((cost benefit) OR (cost benefit):kw)) OR ((cost effectiveness) OR (cost effectiveness):kw) OR (MeSH descriptor Cost-Benefit Analysis explode all trees) OR (MeSH descriptor Costs and Cost Analysis explode all trees)) AND <strong>Limits</strong> [humans]/lim AND [english]/lim</td>
</tr>
</tbody>
</table>
APPENDIX 5 – Tools for assessing the evidence in the systematic review

Table A5.1: NHMRC Dimensions of Evidence

<table>
<thead>
<tr>
<th>Type of evidence</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strength of the evidence</td>
<td>The study design used, as an indicator of the degree to which bias has been eliminated by design.</td>
</tr>
<tr>
<td></td>
<td>The methods used by investigators to minimise bias within a study design.</td>
</tr>
<tr>
<td></td>
<td>The p-value or, alternatively, the precision of the estimate of the effect (as indicated by the confidence interval). It reflects the degree of certainty about the existence of a true effect.</td>
</tr>
<tr>
<td>Size of effect</td>
<td>The distance of the study estimate from the “null” value and the inclusion of only clinically important effects in the confidence interval.</td>
</tr>
<tr>
<td>Relevance of evidence</td>
<td>The usefulness of the evidence in clinical practice, particularly the appropriateness of the outcome measures used.</td>
</tr>
</tbody>
</table>

Table A5.2: NHMRC designations of levels of evidence for an intervention

<table>
<thead>
<tr>
<th>Level</th>
<th>Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>A systematic review of level II studies</td>
</tr>
<tr>
<td>II</td>
<td>A randomised controlled trial</td>
</tr>
<tr>
<td>III-1</td>
<td>A pseudo randomised controlled trial (i.e. alternate allocation or some other method)</td>
</tr>
<tr>
<td>III-2</td>
<td>A comparative study with concurrent controls:</td>
</tr>
<tr>
<td></td>
<td>• Non-randomised, experimental trial</td>
</tr>
<tr>
<td></td>
<td>• Cohort study</td>
</tr>
<tr>
<td></td>
<td>• Case-control study</td>
</tr>
<tr>
<td></td>
<td>• Interrupted time series with a control group</td>
</tr>
<tr>
<td>III-3</td>
<td>A comparative study without concurrent controls:</td>
</tr>
<tr>
<td></td>
<td>• Historical control study</td>
</tr>
<tr>
<td></td>
<td>• Two or more single arm study</td>
</tr>
<tr>
<td></td>
<td>• Interrupted time series without a parallel control group</td>
</tr>
<tr>
<td>IV</td>
<td>Case series with either post-test or pre-test/post-test outcomes</td>
</tr>
</tbody>
</table>

Source: Hierarchies adapted and modified from: NHMRC 1999; Bandolier 1999; Lijmer et al. 1999; Phillips et al. 2001
### Table A5.3: NHMRC quality criteria for RCTs, cohort studies, case-control studies and systemic reviews

<table>
<thead>
<tr>
<th>Study type</th>
<th>Quality criteria</th>
</tr>
</thead>
</table>
| **Randomised controlled trials**    | Was the study double blinded?  
|                                     | Was allocation to treatment groups concealed from those responsible for recruiting the subjects?  
|                                     | Were all randomised participants included in the analysis?  
| **Cohort studies**                  | How were subjects selected for the “new intervention”?  
|                                     | How were subjects selected for the comparison or control group?  
|                                     | Does the study adequately control for demographic characteristics, clinical features and other potential confounding variables in the design or analysis?  
|                                     | Was the measurement of outcomes unbiased (i.e. blinded to treatment group and comparable across groups)?  
|                                     | Was follow-up long enough for outcomes to occur?  
|                                     | Was follow-up complete and were there exclusions from the analysis?  
| **Case-control studies**            | How were cases defined and selected?  
|                                     | How were controls defined and selected?  
|                                     | Does the study adequately control for demographic characteristics and important potential confounders in the design or analysis?  
|                                     | Was measurement of exposure to the factor of interest (e.g. the new intervention) adequate and kept blinded to case/control status?  
|                                     | Were all selected subjects included in the analysis?  
| **Systematic reviews**              | Was an adequate search strategy used?  
|                                     | Were the inclusion criteria appropriate and applied in an unbiased way?  
|                                     | Was a quality assessment of included studies undertaken?  
|                                     | Were the characteristics and results of the individual studies appropriately summarised?  
|                                     | Were the methods for pooling the data appropriate?  
|                                     | Were sources of heterogeneity explored?  

Source: National Health and Medical Research Council (NHMRC), 2000. How to review the evidence: systematic identification and review of the scientific literature, NHMRC, Commonwealth of Australia, Canberra.


b Based on quality assessment instruments developed and being tested in Australia and Canada.

c Based on articles by Greenhalgh (1997) and Hunt and McKibbon (1997).