Vitamin B12 and Methylmalonic Acid Testing

Policy Number: APEA – G2014 – Vitamin B12 and Methylmalonic Acid Testing
Initial Presentation Date: 7/01/2020
Revision Date: 7/01/2020

Policy Description

Vitamin B12, also known as cobalamin, is a water-soluble vitamin required for proper red blood cell formation, key metabolic processes, neurological function, and DNA regulation and synthesis. Hematologic and neuropsychiatric disorders caused by a deficiency in B12 can often be reversed by early diagnosis and prompt treatment (Oh & Brown, 2003).

Methylmalonic acid is produced from excess methylmalonyl-CoA that accumulates when Vitamin B12 is unavailable and is considered an indicator of functional B12 deficiency (Sobczynska-Malefora et al., 2014).

Holotranscobalamin is the metabolically active fraction of B12 and is an emerging marker of impaired vitamin B12 status (Langan & Goodbred, 2017).

Related Policies

<table>
<thead>
<tr>
<th>Policy Number</th>
<th>Policy Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>APEA-G2154</td>
<td>Folate Testing</td>
</tr>
<tr>
<td>AHA-M2141</td>
<td>Testing of Homocysteine Metabolism-Related Conditions</td>
</tr>
</tbody>
</table>

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual’s benefit coverage at the time of the request.

1. Vitamin B12 testing MEETS COVERAGE CRITERIA in individuals being evaluated for clinical manifestations of Vitamin B12 deficiency including:
   a. Cutaneous
      i. Hyperpigmentation
      ii. Jaundice
      iii. Vitiligo
   b. Gastrointestinal
i. Glossitis

c. Hematologic
   i. Anemic (macrocytic, megaloblastic)
   ii. Leukopenia
   iii. Pancytopenia
   iv. Thrombocytopenia
   v. Thrombocytosis

d. Neuropsychiatric
   i. Areflexia
   ii. Cognitive impairment (including dementia-like symptoms and acute psychosis)
   iii. Gait abnormalities
   iv. Irritability
   v. Loss of proprioception and vibratory sense
   vi. Olfactory impairment
   vii. Peripheral neuropathy

2. Vitamin B12 testing **MEETS COVERAGE CRITERIA** when performed no sooner than 3 months after initiation of therapy for individuals undergoing treatment for vitamin B12 deficiency.

3. Screening for Vitamin B12 deficiency **MEETS COVERAGE CRITERIA** for individuals with one or more of the following risk factors:
   
a. Decreased ileal absorption
      i. Crohn disease
      ii. Ileal resection
      iii. Tapeworm infection
   
b. Genetic
      i. Transcobalamin II deficiency
   
c. Inadequate intake
      i. Alcohol abuse
      ii. Patients older than 75 years or elderly individuals being evaluated for dementia
      iii. Vegans or strict vegetarians (including exclusively breastfed infants of vegetarian/vegan mothers)
      iv. Eating disorders
d. Prolonged medication use
   i. Histamine H2 blocker use for more than 12 months
   ii. Metformin use for more than four months
   iii. Proton pump inhibitor use for more than 12 months

4. Screening for Vitamin B12 deficiency in healthy, asymptomatic individuals **DOES NOT MEET COVERAGE CRITERIA.**

5. Methylmalonic acid testing **MEETS COVERAGE CRITERIA** to confirm vitamin B12 deficiency in asymptomatic high-risk patients with low-normal levels of vitamin B12 or when vitamin B12 deficiency is suspected but the serum vitamin B12 level is normal or low-normal.

6. Methylmalonic acid **MEETS COVERAGE CRITERIA** for the evaluation of inborn errors of metabolism, which is out of scope for this policy.

7. Homocysteine testing **DOES NOT MEET COVERAGE CRITERIA** for the confirmation of vitamin B12 deficiency.

8. Holotranscobalamin testing **DOES NOT MEET COVERAGE CRITERIA** for the screening, testing or confirmation of vitamin B12 deficiency.

**Scientific Background**

Vitamin B12 cannot be synthesized by human cells (Schrier, 2017), rather it is obtained from animal derived dietary sources, such as meat, eggs and dairy products (Hunt, Harrington, & Robinson, 2014), as well as fortified cereals and supplements (Zeuschner et al., 2013). Vitamin B12 deficiency is classically caused by pernicious anemia; however, with modern fortification of western diets, this condition now accounts for only a minority of cases and currently occurs most often due to malabsorption (Schrier, 2017). Reflecting this, the prevalence of vitamin B12 deficiency in the United States and United Kingdom is approximately 6% in persons younger than 60 years, reaching 20% in those older than 60 years. On the contrary, the prevalence is approximately 40% in Latin America, 70% in Kenyan school children, 80% in East Indian preschool-aged children, and 70% in East Indian adults (Hunt et al., 2014). Risk factors for deficiency include (Langan & Goodbred, 2017): decreased ileal absorption (Crohn disease, ileal resection, tapeworm infection), decreased intrinsic factor (atrophic gastritis, pernicious anemia, post-gastrectomy syndrome), genetic defects (transcobalamin II deficiency), inadequate intake (alcohol abuse, patients older than 75 years, vegans or strict vegetarians), prolonged medication use (histamine H2 blocker use for more than 12 months, metformin use for more than four months, proton pump inhibitor use for more than 12 months).

Vitamin B12 plays an essential role in nucleic acid synthesis, and deficiency can result in cell cycle arrest in the S phase or apoptosis (Green, 2017) and ultimately bone marrow failure and demyelinating nervous system disease (Stabler, 2013). Clinical manifestations vary in their presence and severity (Langan & Goodbred, 2017) from mild fatigue to severe neurologic impairment. Mild deficiency can present as fatigue and anemia with an absence of neurological features. Moderate deficiency may include obvious macrocytic anemia with some mild or subtle neurological features. Severe deficiency shows evidence of bone marrow suppression, clear evidence of neurological features, and risk of cardiomyopathy. Early detection and correction of vitamin B12 deficiency with supplementation prevents progression to macrocytic anemia, elevated homocysteine, potentially irreversible peripheral neuropathy, memory loss, and other cognitive deficits (Sobczynska-Malefora et al., 2014).

**Analytical Validity**

Both the clinical recognition of vitamin B12 deficiency and confirmation of the diagnosis by
Means of testing can be difficult. Several laboratory measures reflecting physiological, static, and functional B12 status have been developed (Hunt et al., 2014); however, there is no universally agreed upon gold standard assay for determining cobalamin levels in humans. The current convention is to estimate the abundance of vitamin B12 using total serum vitamin B12, despite the low sensitivity of this test. (Sobczynska-Malefora et al., 2014). Two reportedly highly sensitive vitamin B12 deficiency markers are elevated levels of serum homocysteine and methylmalonic acid; however, testing is expensive, and many other conditions may cause an elevation in these markers, including homocysteine, familial hyperhomocysteinemia, folate deficiency, levodopa therapy, and renal insufficiency (Langan & Zawistoski, 2011). Serum methylmalonic acid levels tend to be just as sensitive but more specific than serum homocysteine levels in regards to vitamin B12 deficiency testing, highlighting the former as the preferred testing method by many (Langan & Zawistoski, 2011).

An in-depth meta-analysis by Willis and colleagues of serum cobalamin testing included data from 54 different studies. The variability for sensitivity and specificity across the different studies ranged from 13% to 75% for sensitivity and 45% to 100% for specificity, depending on the reference standard used. Researchers conclude that “from the available evidence, diagnosis of conditions amenable to cbl [vitamin B12] supplementation on the basis of cbl [vitamin B12] level alone cannot be considered a reliable approach to investigating suspected vitamin deficiency” (Willis et al., 2011). The test measures total serum cobalamin including both serum holohaptocorrin and serum holotranscobalamin, which may mask true deficiency or falsely imply a deficient state (Hunt et al., 2014).

Vitamin B12 deficiency is present in both infant and pregnant female populations, and monitoring vitamin B12 levels is important in determining maternal and fetal health and growth; low vitamin B12 levels during pregnancy are associated with a greater risk of preterm birth (Rogne et al., 2017). It seems that current pregnancy-specific cutoffs for vitamin B12 biomarkers are inadequate in the medical field (Schroder et al., 2019). Recently, a new study has identified a novel cutoff value in the vitamin B12 serum of newborns; the B12-related metabolite known as homocysteine (Hcy) cutoff value is now recommended at “4.77 µmol/L (68.4% sensitivity, 58.3% specificity, p = .012) for the detection of vit-B12 deficiency” (Yetim et al., 2019). Other pregnancy-specific B12 biomarkers have been published. According to another study, "The central 95% reference interval limits indicated that serum total B-12 <89.9 and <84.0 pmol/L, holoTC <29.5 and <26.0 pmol/L and MMA >371 and >374 nmol/L, in the first and second trimesters, respectively, may indicate B-12 deficiency in pregnant women. The lower limits of total B-12 and holoTC and the upper limits of MMA significantly differed by ethnicity in both trimesters. According to the change point analysis, total B-12 <186 and <180 pmol/L and holoTC <62.2 and <67.5 pmol/L in the first and second trimesters, respectively, suggested an increased probability of impaired intracellular B-12 status, with no difference between ethnicities (Schroder et al., 2019)."

Elevated levels of downstream metabolites, methylmalonic acid (MMA) and homocysteine, are commonly used as adjuvant diagnostics to confirm a suspected diagnosis of cobalamin deficiency (Berg & Shaw, 2013). The sensitivity of elevated serum MMA measurements in detecting patients with overt cobalamin deficiency is reported to be >95%; however, the specificity of this test has not been determined (Hunt et al., 2014). Serum holotranscobalamin may be a better indicator of B12-deficiency than serum cobalamin because it represents the biologically active fraction of cobalamin in humans and may be depleted first in subclinical cobalamin deficiency. Holotranscobalamin measurements appear to have slighter better sensitivity; however, the specificity of this assay remains to be determined (Oberley & Yang, 2013). It also is not yet clinically validated or available for widespread use (Langan & Goodbred, 2017).

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Pitfalls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum cobalamin (&lt;200 pg/mL)</td>
<td>95–97%</td>
<td><strong>Uncertain, possibly &lt;80%</strong></td>
<td><strong>Elevated levels seen with:</strong> Assay technical failure Occult malignancy</td>
</tr>
<tr>
<td>Criteria</td>
<td>Sensitivity</td>
<td>Specificity</td>
<td>Pitfalls</td>
</tr>
<tr>
<td>------------------------------</td>
<td>-------------</td>
<td>--------------------------------</td>
<td>---------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Elevated serum methylmalonic acid</td>
<td>&gt;95%</td>
<td>Uncertain</td>
<td>Elevated levels seen with:</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Renal insufficiency</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hypovolemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Congenital metabolic defects</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Amyotrophic lateral sclerosis</td>
</tr>
<tr>
<td>Decreased serum homocysteine</td>
<td>&gt;95%</td>
<td>Uncertain, less specific than methylmalonic acid</td>
<td>Elevated levels seen with:</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Folate or pyridoxine deficiency</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Renal insufficiency</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hypovolemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hypothyroidism</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Psoriasis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Congenital metabolic defects</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Neurodegenerative disease</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Malignancy</td>
</tr>
<tr>
<td>Decreased serum holotranscobalamin</td>
<td>Similar to total cobalamin</td>
<td>Uncertain</td>
<td>Levels may be affected by:</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Liver disease</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Macrophage activation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Autoantibodies</td>
</tr>
</tbody>
</table>

**Clinical Utility and Validity**

Health Quality Ontario performed an extensive meta-analysis of the clinical utility of B12 testing in patients with suspected dementia or cognitive decline because more than 2.9 million serum B12 tests were performed in Ontario alone in 2010 (HQO, 2013). HQO included data from eighteen different studies to address three questions: “Is there an association between vitamin B12 deficiency and the onset of dementia or cognitive decline? Does treatment with vitamin B12 supplementation improve cognitive function in patients with dementia or cognitive decline and
vitamin B12 deficiency? What is the effectiveness of oral versus parenteral vitamin B12 supplementation in those with confirmed vitamin B12 deficiency?” They concluded that “This evidence-based analysis assessed the usefulness of serum vitamin B12 testing as it relates to brain function. This review found very low quality evidence that suggests a connection between high plasma homocysteine levels (a by-product of B vitamin metabolism in the body) and the onset of dementia. Moderate quality of evidence indicates treatment with vitamin B12 does not improve brain function. Moderate quality of evidence also indicates treatment using oral vitamin B12 supplements is as effective as injections of vitamin B12 (HQO, 2013).”

Another meta-analysis, completed in 2015, utilized data from 12 studies and a total of 34,481 patients to determine if vitamin B12, vitamin B6, and folic acid supplementation affected homocysteine levels and/or reduced the risk of cardiovascular disease (Li, Li, Qi, & Shen, 2015). A combination of vitamin B12, vitamin B6, and folic acid was found to significantly reduce plasma homocysteine levels, but it did not seem to impact cardiovascular disease risk (Li et al., 2015). Therefore, it was concluded that vitamin B12 should not be utilized as a cardiovascular disease prevention method. Additional research has also concluded that the “Use of vitamin B12 in patients with elevated serum homocysteine levels and cardiovascular disease does not reduce the risk of myocardial infarction or stroke, or alter cognitive decline (Langan & Goodbred, 2017).”

In other indications, vitamin B12 has recently been utilized as a biomarker for patients undergoing therapeutic treatment for tuberculosis; in particular, vitamin B12 serum concentrations were utilized to monitor treatment responses (Gebremicael et al., 2019).

Guidelines and Recommendations

American Academy of Family Physicians (AAFP) (Langan & Goodbred, 2017)
The AAFP does not recommend screening persons at average risk of vitamin B12 deficiency. Screening should be considered in patients with risk factors, and diagnostic testing should be considered in those with suspected clinical manifestations.

“The recommended laboratory evaluation for patients with suspected vitamin B12 deficiency includes a complete blood count and serum vitamin B12 level. In patients with a normal or low-normal serum vitamin B12 level, complete blood count results demonstrating macrocytosis, or suspected clinical manifestations, a serum methylmalonic acid level is an appropriate next step and is a more direct measure of vitamin B12’s physiologic activity; although not clinically validated or available for widespread use, measurement of holotranscobalamin, the metabolically active form of vitamin B12, is an emerging method of detecting deficiency (Langan & Goodbred, 2017).”

American College of Gastroenterology (ACG) (Rubio-Tapia, Hill, Kelly, Calderwood, & Murray, 2013)
According to the ACG, “people with newly diagnosed celiac disease should undergo testing and treatment for micronutrient deficiencies. Deficiencies to be considered for testing should include, but not be limited to, iron, folic acid, vitamin D, and vitamin B12 (conditional recommendation, low level of evidence) (Rubio-Tapia et al., 2013).”

American Academy of Neurology (AAN) (Knopman et al., 2001)
“The American Academy of Neurology recommends serum vitamin B12 testing as part of the assessment of elderly patients with dementia (Knopman et al., 2001).”

British Committee for Standards in Haematology (Devalia, Hamilton, & Molloy, 2014)
“Serum cobalamin currently remains the first-line test, with additional second-line plasma methylmalonic acid to help clarify uncertainties of underlying biochemical/functional deficiencies. Serum holotranscobalamin has the potential as a first-line test, but an indeterminate ‘grey area’ may still exist. Plasma homocysteine may be helpful as a second-line test but is less specific than methylmalonic acid. The availability of these second-line tests is
currently limited (Devalia et al., 2014).”

**The Doctors of BC (formerly the British Columbia Medical Association) (BCMA, 2013)**
The doctors of BC recommend vitamin B12 testing for individuals with “unexplained neurologic symptoms such as paresthesias, numbness, poor motor coordination, memory lapses, or cognitive and personality changes,” and anemia. They also recommend consideration of testing of elderly individuals (>75 years old), those with inflammatory bowel disease (of small intestine), gastric or small intestine resection, prolonged vegan diet, and long-term use of H2 receptor antagonists or proton pump inhibitors (at least 12 months), or metformin (at least 4 months).

**American Association of Clinical Endocrinologists (AACE) and the American College of Endocrinology (ACE) and the Obesity Society (Gonzalez-Campoy et al., 2013)**
“Vitamin B12 levels should be checked periodically in older adults and patients on metformin therapy (Grade A, BEL 1). With the exception of early treatment of patients with neurologic symptoms, pernicious anemia, or malabsorptive bariatric surgery requiring parenteral (intramuscular or subcutaneous) vitamin B12 replacement, patients with vitamin B12 deficiency can generally be treated with oral vitamin B12 (1,000 μg per day of oral crystalline cobalamin) and may benefit from increasing the intake of vitamin B12 in food (Grade A, BEL 1) (Gonzalez-Campoy et al., 2013).”

**American Association of Clinical Endocrinologists (AACE), the Obesity Society, and American Society for Metabolic & Bariatric Surgery (ASMBS) (Mechanick et al., 2013)**
“Baseline and postoperative evaluation for vitamin B12 deficiency is recommended in all bariatric surgery and annually in those with procedures that exclude the lower part of the stomach (e.g., LSG, RYGB) (Grade B; BEL 2) (Mechanick et al., 2013).”

Concerning vitamin B12 screening and weight loss surgical (WLS) practices, the ASMBS states that “routine pre-WLS screening of B12 is recommended for all patients (Grade B, BEL 2).” Further, serum MMA testing is recommended to evaluate a possible B12 deficiency for both asymptomatic and symptomatic patients as well as in “those with history of B12 deficiency or preexisting neuropathy (Grade B, BEL 2) (Parrott et al., 2017).”

**Guidelines for Diagnosis and Management of the Cobalamin-related Remethylation Disorders cblC, cblD, cblE, cblF, cblG, cblJ and MTHFR Deficiency (Huemer et al., 2017)**
This international consortium of scientists from Europe and the U.S. issued guidelines “within the frame of the ‘European network and registry for homocystinurias and methylation defects’ (E-HOD) project.” For Recommendation 5, they state (Quality of the evidence: moderate), “we strongly recommend that in the case of high total homocysteine, plasma and urine samples for determination of MMA, methionine, folate and vitamin B12 are to be obtained before treatment is started (Huemer et al., 2017).”

**The American Diabetic Association (ADA) (ADA, 2019)**
The ADA states that in patients with type 2 diabetes, the long-term use of metformin may be associated with a vitamin B12 deficiency; therefore, a recommendation has been made which states that the “periodic measurement of vitamin B12 levels should be considered in metformin-treated patients, especially in those with anemia or peripheral neuropathy” (ADA, 2019).

**American Psychiatric Association (APA) (Yager et al., 2006)**
The APA released guidelines which include a table of the physical complications of anorexia nervosa and potential laboratory testing methods. This table contains a few vitamin assays that may be used to monitor endocrine or metabolic processes including vitamin B12 assays “in severe cases” (Yager et al., 2006).
U.S. Preventative Services Task Force (USPSTF) (Langan & Zawistoski, 2011)
Currently, the USPSTF has not published guidelines for vitamin B12 deficiency screenings of asymptomatic or low-risk adults.

State and Federal Regulations, as applicable

A search of the FDA Device database on 10/03/2019 using the term “vitamin B12” yielded 42 results. Additionally, many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA ’88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes

<table>
<thead>
<tr>
<th>Code Number</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>82607</td>
<td>Cyanocobalamin (Vitamin B-12)</td>
</tr>
<tr>
<td>83090</td>
<td>Homocysteine</td>
</tr>
<tr>
<td>83921</td>
<td>Organic acid, single, quantitative</td>
</tr>
<tr>
<td>84999</td>
<td>Unlisted chemistry procedure</td>
</tr>
</tbody>
</table>


Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

Evidence-based Scientific References


**Policy Implementation/Update Information**

7/1/20  New Policy

State and Federal mandates and health plan contract language, including specific provisions/exclusions, take precedence over Medical Policy and must be considered first in determining eligibility for coverage. The medical policies contained herein are for informational purposes. The medical policies do not constitute medical advice or medical care. Treating health care providers are independent contractors and are neither employees nor agents of Blue KC and are solely responsible for diagnosis, treatment and medical advice. No part of this publication may be reproduced, stored in a retrieval system or transmitted, in any form or by any means, electronic, photocopying, or otherwise, without permission from Blue KC.