Name of Policy: Serum Holotranscobalamin as a Marker of Vitamin B12 (Cobalamin) Status

Policy #: 448
Category: Medicine/Laboratory

Latest Review Date: August 2013
Policy Grade: Effective 08/29/2013:
Active Policy but no longer scheduled for regular literature reviews and updates.

Background/Definitions:
As a general rule, benefits are payable under Blue Cross and Blue Shield of Alabama health plans only in cases of medical necessity and only if services or supplies are not investigational, provided the customer group contracts have such coverage.

The following Association Technology Evaluation Criteria must be met for a service/supply to be considered for coverage:

1. The technology must have final approval from the appropriate government regulatory bodies;
2. The scientific evidence must permit conclusions concerning the effect of the technology on health outcomes;
3. The technology must improve the net health outcome;
4. The technology must be as beneficial as any established alternatives;
5. The improvement must be attainable outside the investigational setting.

Medical Necessity means that health care services (e.g., procedures, treatments, supplies, devices, equipment, facilities or drugs) that a physician, exercising prudent clinical judgment, would provide to a patient for the purpose of preventing, evaluating, diagnosing or treating an illness, injury or disease or its symptoms, and that are:

1. In accordance with generally accepted standards of medical practice; and
2. Clinically appropriate in terms of type, frequency, extent, site and duration and considered effective for the patient’s illness, injury or disease; and
3. Not primarily for the convenience of the patient, physician or other health care provider; and
4. Not more costly than an alternative service or sequence of services at least as likely to produce equivalent therapeutic or diagnostic results as to the diagnosis or treatment of that patient’s illness, injury or disease.
**Description of Procedure or Service:**
Holotranscobalamin (holo-TC) is a transcobalamin-vitamin B12 complex which has been investigated as a diagnostic test for vitamin B12 deficiency in symptomatic and at-risk populations, as well as an assay for monitoring response to therapy.

Vitamin B12 (cobalamin) is an essential vitamin that is required for DNA synthesis affecting red blood cell formation, and methionine synthesis affecting neurological functioning. Cobalamin deficiency can result from nutritional deficiencies or malabsorption. Dietary insufficiency is most common among vegetarians and the elderly. Malabsorption of vitamin B12 may be associated with autoantibodies as in pernicious anemia or can occur after gastrectomy, or in other gastrointestinal conditions such as celiac disease, Whipple’s disease and Zollinger-Ellison syndrome. Clinical signs and symptoms of cobalamin deficiency include megaloblastic anemia, paresthesias and neuropathy, and psychiatric symptoms such as irritability, dementia, depression, or psychosis. While the hematologic abnormalities disappear promptly after treatment, neurologic disorders may become permanent if treatment is delayed.

The diagnosis of cobalamin deficiency has traditionally been based on low levels of total serum cobalamin, typically less than 200 pg/ml, in conjunction with clinical evidence of disease. However, this laboratory test has been found to be poorly sensitive and specific. Therefore, attention has turned to measuring metabolites of cobalamin as a surrogate marker. For example, in humans only two enzymatic reactions are known to be dependent on cobalamin: the conversion of methylmalonic acid (MMA) to succinyl-CoA, and the conversion of homocysteine and folate to methionine. Therefore, in the setting of cobalamin deficiency, serum levels of MMA and homocysteine are elevated, and have been investigated as surrogate markers.

There also is interest in the direct measurement of the subset of biologically-active cobalamin. Cobalamin in serum is bound to two proteins, transcobalamin and haptocorrin. Transcobalamin-cobalamin complex (called holotranscobalamin, or holo-TC) functions to transport cobalamin from its site of absorption in the ileum to specific receptors throughout the body. Less than 25% of the total serum cobalamin exists as holo-TC, but this is considered the clinically relevant biologically active form. Serum levels of holo-TC can be measured using a radioimmunoassay or enzyme immunoassay.

**Policy:**
**Measurement of holotranscobalamin does not meet** Blue Cross and Blue Shield of Alabama’s medical criteria for coverage and is considered **investigational** in the diagnosis and management of Vitamin B12 deficiency.

*Blue Cross and Blue Shield of Alabama does not approve or deny procedures, services, testing, or equipment for our members. Our decisions concern coverage only. The decision of whether or not to have a certain test, treatment or procedure is one made between the physician and his/her patient. Blue Cross and Blue Shield of Alabama administers benefits based on the members' contract and corporate medical policies. Physicians should always exercise their best medical judgment in providing the care they feel is most appropriate for their patients. Needed care should not be delayed or refused because of a coverage determination.*
**Key Points:**
There were no clinical trials identified that directly evaluated the utility of testing cobalamin status with serum holotranscobalamin. There were also no trials that evaluated the benefit of treatment in individuals with subclinical cobalamin deficiency. The diagnostic performance and operating characteristics continue to be an area of active research. One systematic review and several randomized controlled trials (RCTs) have been identified addressing this area.

Validation of the clinical use of any diagnostic test focuses on 3 main principles: 1) the technical feasibility of the test; 2) the diagnostic performance of the test, such as sensitivity, specificity, and positive and negative predictive value in different populations of patients and compared to the gold standard; and 3) the clinical utility of the test, i.e., how the results of the diagnostic test will be used to improve the management of the patient.

**Technical Feasibility**
The serum measurements of holo-TC involve the use of standard laboratory immunoassay techniques. In the first step, holo-TC in the serum sample is separated by magnetic microspheres coated with monoclonal antibodies to human transcobalamin. The cobalamin bound to the holo-TC is then released and measured by a competitive binding radioimmunoassay or fluorescence, depending on the device used.

**Diagnostic Performance**
The diagnostic performance must be compared to the established gold standard for measuring cobalamin deficiency. This is particularly problematic, since there is currently no established gold standard. As noted in the Description section, serum levels of total cobalamin are considered poorly sensitive and specific, and holo-TC measurements are not independent of total cobalamin measures, leading to a potential bias in the estimate of the test’s diagnostic power. There have been several reports proposing serum measures of methylmalonic acid (MMA) and homocysteine as an alternative gold standard; their specificity has been questioned.

According to the FDA decision summary, the cut-off values for holo-TC were based on a normal population instead of a population of those with known cobalamin deficiency. For example, the low value of holo-TC, 37 pmol/L, was based on a study of 303 normal Finnish individuals. This study has also been published by Loikas et al in the peer-reviewed literature. Participants included 226 normal elderly subjects and 80 normal, non-elderly adult subjects. Patients were excluded from the trial if they had hyperhomocysteinemia, evidence of a possible cobalamin deficiency. In addition, patients in the lowest one third of holo-TC results underwent additional testing with methylmalonic acid (MMA); those with elevated MMA levels were also excluded. In the normal reference population, the holo-TC range was 25–254 pmol/L with a 95% central reference interval of 37–171 pmol/L. Therefore, the cut-off value for a low result was established at 37 pmol/L. This cut-off value was then applied to the results of 107 patients with presumed cobalamin deficiency, as evidenced by different combinations of an increased plasma homocysteine or MMA level, or a low total serum cobalamin level, defining patients with potential, possible, or probable cobalamin deficiency. A total of 48% of those with presumed deficiency had a holo-TC below 37 pmol/L. The frequencies of low holo-TC levels increased with increasing pretest probability of cobalamin deficiency. For example, among the 16 patients thought to have the highest pretest probability of cobalamin deficiency, based on elevated levels...
of homocysteine and MMA, 100% had low levels of holo-TC. Therefore, this study used levels of homocysteine and MMA as the gold standard. Based on this standard, the sensitivity of the test was only 48% among those with cobalamin deficiency rated as either potential, possible, or probable. The authors conclude that further studies are needed to confirm the clinical utility and specificity of holo-TC in diagnosis of subclinical cobalamin deficiency. Also, these values for a homogeneous population of Finnish subjects with a diet high in fish might not be able to be extrapolated to the heterogeneous American population and diet. Furthermore, these cut-off points require confirmation in a larger population of patients whose cobalamin status is unknown.

In 2013 Dullemeijer et al. reported on a systematic review and meta-analysis of studies on biomarker responses to B12 supplementation. The authors found doubling the intake of B12 increased serum or plasma levels of B12 by 11% and decreased MMA levels by 7%. However, only two small RCTs with three holo-TC estimates were identified which showed B12 supplementation significantly increased serum or plasma holo-TC levels. However, the small size of the RCTs precluded meta-analysis. The authors cautioned the heterogeneity of studies limited the interpretation of the results reported.

O’Leary and colleagues in 2012 reported on a systematic review of B12 status and its relationship to cognitive decline and dementia. The authors evaluated 35 cohort studies and found serum B12 levels were not associated with cognitive decline or dementia. However, four studies found increased risks of cognitive decline or dementia were associated with MMA and/or holo-TC levels. Nevertheless, the use of underpowered cohort studies of short duration limits interpretation of these results.

In April 2009, Hoey et al published a systematic review of the response of various biomarkers to treatment with vitamin B12. Only one RCT by Eussen and colleagues utilizing holo-TC was identified for the review, therefore the authors concluded that data were insufficient to draw conclusions about the effectiveness of serum holo-TC as a biomarker for vitamin B12 status.

In 2013, Hill and colleagues reported on a double-blind, placebo-controlled, randomized study of 100 elderly patients with poor B12 status. Patients were treated for eight weeks with vitamin B12 supplements of 10 μg/d, 100 μg/d, or 500 μg/d. Compared to placebo, all B12 dosages had an effect on holo-TC levels (p< 0.01). However, even after receiving 500 μg/d B12 for 56 days, 12% of patients had below threshold (>200pmol/L) plasma B12 levels and 56% still had elevated plasma and urine MMA levels suggesting continued metabolic insufficiency despite supplementation.

In a double-blind trial to determine the effects of B12 supplementation of cognitive functioning in older adults, Eussen et al measured holo-TC at baseline, 12, and 24 weeks in 195 subjects randomized to three groups: cobalamin, cobalamin plus folate supplementation, or placebo. The primary outcome measure was cognitive improvement. The results did not support a significant difference in cognitive functioning. The authors noted a significant time-treatment interaction after 12 weeks in both treatment arms of holo-TC for all biomarkers measured (vitamin B12, MMA, holo-TC, homocysteine and red blood cell folate [p<0.0002]). Specifically for holo-TCA, in the vitamin B12 group, mean levels increased from 58 + 21 to 183 + 124 (p<0.05 for
difference from baseline). In the folate and vitamin B12 supplementation group, holo-TC increased from 68 ± 33 to 222 ± 133 (p<0.05 for difference from baseline). Comparatively, the placebo group’s levels did not significantly change, from 70 ± 39 to 65 ± 43 (p<0.05 for difference from treatment groups). Further changes did not occur between 12 and 24 weeks of supplementation.

Eussen and colleagues published a smaller trial in 2008. Once again, patients were randomized to cobalamin, cobalamin plus folate, or placebo supplementation in subjects with known mild cobalamin deficiency. Along with serum cobalamin and MMA levels, holo-TC was utilized to assess deficiency status, and did rise in response to therapy. Other recent studies have utilized holo-TC as one of a number of measures of cobalamin status. However, these studies do not attempt to assess the independent predictive capacity of the test, and therefore do not add to the evidence base for this policy.

Valente and colleagues reported on the diagnostic accuracy of holotranscobalamin, MMA, serum cobalamin, and other indicators of tissue vitamin B12 status in an elderly population. Elderly subjects (n=700), age range 63-97 years, were recruited from an ongoing observational cohort study to collect data on 2,000 individuals older than 60 years with mild to moderate cognitive impairment. A separate reference population of 120 healthy volunteers, age 18-62 years, was used to determine a reference interval for the red cell cobalamin assay. The cut-offs for deficiency were defined as 20 pmol/L for holo-TC, 123 pmol/L for serum cobalamin, and less than 33 pmol/L for red cell cobalamin. The red cell lower limit of 33 pmol/L packed red cells was used to dichotomize the concentrations into deficient and nondeficient vitamin B12 status for the construction of receiver operating characteristic (ROC) plots. The areas under the curve (AUC) showed that serum holo-TC was the best predictor with AUC 0.90 (95% confidence interval [CI]: 0.86-0.93), and this was significantly better (p<0.0002) than the next best predictors of serum cobalamin 0.80 (95% CI: 0.75-0.85), and MMA 0.78 (95% CI: 0.72-0.83). For these three analytes, the authors constructed a 3-zone partition of positive and negative zones and a deliberate indeterminate zone between. The boundaries were values of each test that resulted in a post-test probability of deficiency of 60% and a post-test probability of no deficiency of 98%. The proportion of indeterminate observations for holo-TC, cobalamin, and MMA was 14%, 45%, and 50%, respectively.

Clinical Utility
Advocates of holo-TC testing suggest that this laboratory test can identify early subclinical stages of cobalamin deficiency or other conditions, permitting prompt initiation of treatment, specifically supplementary cobalamin dietary supplementation. Further, this reasoning suggests that early diagnosis will lead to an improvement in health outcome in patients. This hypothesis was not directly tested in any of the identified published literature. In the absence of a gold standard, the clinical significance of subclinical cobalamin deficiency must be further studied by understanding the natural history of this condition. Does subclinical deficiency inevitably progress to clinical deficiency? Does cobalamin supplementation normalize the values? How variable are cobalamin levels within patients? These clinical issues have not been well addressed in the literature. Finally, for all patients at risk, i.e., vegetarians, the elderly, and post-gastrectomy patients, the recommended treatment of subclinical disease is further dietary
supplementation of cobalamin. This recommendation is appropriate, regardless of the level of measured cobalamin.

Heil and colleagues aimed to validate the clinical usefulness of holo-TC as an initial screening assay for metabolic vitamin B12 deficiency in a mixed patient population. Three hundred and sixty blood samples were collected by five Dutch hospitals, and vitamin B12 and holo-TC in serum were measured. MMA in serum was measured by tandem mass spectrometry. Receiver-operating-curve analysis demonstrated a greater area under the curve for holo-TC than for vitamin B12 in detecting vitamin B12 deficiency characterized by three predefined cut-off levels of MMA. A cut-off value of 32 pmol/L of holo-TC resulted in the highest sensitivity (83%) with acceptable specificity (60%) in detecting MMA concentrations above 0.45 μmol/L. The combination of vitamin B12 and holo-TC did not improve diagnostic accuracy at this cut-off level. The authors concluded that holo-TC has a better diagnostic accuracy than vitamin B12 and can replace the existing vitamin B12 assay as a primary screening test in patients suspected of vitamin B12 deficiency.

Summary
Holotranscobalamin (holo-TC) is a transcobalamin-vitamin B12 complex that has been investigated as a diagnostic test for vitamin B12 deficiency in symptomatic and at-risk populations, as well as an assay for monitoring response to therapy.

There are inadequate data to establish holotranscobalamin testing as an alternative to either total serum cobalamin, or levels of MMA or homocysteine in the diagnosis of vitamin B12 deficiency. While technically feasible, and likely to have diagnostic performance that approaches that of currently utilized tests, no evidence of clinical utility has been demonstrated, neither as a screening tool in the general or at-risk population, nor as a diagnostic tool in symptomatic individuals. Evidence of the clinical utility of the test is currently lacking, and therefore the test remains investigational.

Technology Assessment, Guideline and Position Statements
Many societies have recommended vitamin B12 supplementation for specific clinical conditions or evaluation for vitamin B12 deficiency in the workup for clinical indication without specifying a methodology. An exception is in a practice parameter for peripheral neuropathy by the American Academy of Neurology (AAN), who has specified a methodology (evidence level C): “serum B12 level with metabolites (methylmalonic acid with or without homocysteine)” in the evaluation for vitamin B12 deficiency.

Key Words:
Holo-TC, Vitamin B12 Deficiency, Holotranscobalamin, Transcobalamin, Axis-Shield, HoloTC RIA

Approved by Governing Bodies:
In January 2004, the device “HoloTC RIA” (Axis-Shield plc, Dundee, UK) is an example of a radioimmunoassay for holo-TC that was cleared for marketing by the FDA through the 510(k)
process. The FDA determined that this device was substantially equivalent to existing devices for use in:

"Quantitative measurement of the fraction of cobalamin (vitamin B12) bound to the carrier protein transcobalamin in the human serum or plasma. Measurements obtained by this device are used in the diagnosis and treatment of vitamin B12 deficiency."

In November 2006, the device “Axis-Shield HoloTC Assay” (Axis-Shield, Dundee, UK), an enzyme immunoassay for holo-TC, was cleared for marketing by the FDA through the 510(k) process. The FDA determined that this device was substantially equivalent to existing devices for use in:

"Quantitative determination of holotranscobalamin...in human serum and plasma on the AxSym® System. HoloTC is used as an aid in the diagnosis and treatment of vitamin B12 deficiency."

**Benefit Application:**
Coverage is subject to member’s specific benefits. Group specific policy will supersede this policy when applicable.

ITS: Home Policy provisions apply
FEP: FEP does not consider investigational if FDA approved and will be reviewed for medical necessity.
Pre-certification requirements: Not applicable

**Current Coding:**
CPT Codes:

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<tr>
<th>Code</th>
<th>Description</th>
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<tr>
<td>0103T</td>
<td>Holotranscobalamin, quantitative</td>
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**References:**


Policy History:
Medical Policy Group, September 2010 (3)
Medical Policy Administration Committee, September 2010
Available for comment September 22-November 5, 2010
Medical Policy Group, September 2012 (3): 2012 Update to Key Points and References
Medical Policy Panel, August 2013
Medical Policy Group, August 2013 (1) Update to Key Points and References: no change to policy statement
Effective August 29, 2013: Active Policy but no longer scheduled for regular literature reviews and updates.

This medical policy is not an authorization, certification, explanation of benefits, or a contract. Eligibility and benefits are determined on a case-by-case basis according to the terms of the member’s plan in effect as of the date services are rendered. All medical policies are based on (i) research of current medical literature and (ii) review of common medical practices in the treatment and diagnosis of disease as of the date hereof. Physicians and other providers are solely responsible for all aspects of medical care and treatment, including the type, quality, and levels of care and treatment.

This policy is intended to be used for adjudication of claims (including pre-admission certification, pre-determinations, and pre-procedure review) in Blue Cross and Blue Shield’s administration of plan contracts.