Serum Holotranscobalamin as a Marker of Vitamin B12 (i.e., Cobalamin) Status

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Next Review: February 2024
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IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION

Holotranscobalamin (holoTC) 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.

MEDICAL POLICY CRITERIA

Measurement of holotranscobalamin (holoTC) is considered investigational in the diagnosis and management of Vitamin B12 deficiency.

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

CROSS REFERENCES

None

BACKGROUND

Vitamin B12 (cobalamin) is essential for DNA synthesis affecting red blood cell formation and methionine synthesis affecting neurologic functioning. Cobalamin deficiency can result from
nutritional/dietary deficiencies (most common in the elderly, people with excessive alcohol consumption and people who consume a vegetarian diet), malabsorption of vitamin B12 (seen after gastrectomy or associated with autoantibodies [e.g., pernicious anemia]), or other relatively uncommon gastrointestinal conditions (e.g., Whipple’s disease, Zollinger Ellison syndrome). Clinical signs and symptoms of cobalamin deficiency include megaloblastic anemia, paresthesias and neuropathy, as well as psychiatric symptoms such as irritability, dementia, depression, or psychosis. While the hematologic abnormalities disappear promptly after treatment, neurologic disorders may become permanent if left untreated.

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, the total serum cobalamin laboratory test has poor sensitivity and specificity. Therefore, attention has turned to measuring metabolites of cobalamin as a surrogate marker. For example, in humans two enzymatic reactions are dependent on cobalamin: the conversion of methylmalonic acid (MMA) to succinyl-CoA, and the conversion of homocysteine (Hcy) and folate to methionine. In the setting of cobalamin deficiency, serum levels of MMA and homocysteine are elevated, and have been investigated as surrogate markers.

There is also 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 holoTC) 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 holoTC, but this is considered the clinically relevant biologically active form. Serum levels of holoTC can be measured using standard laboratory immunoassay techniques (i.e., radioimmunoassay or enzyme immunoassay). In the first step, holoTC in the serum sample is separated by magnetic microspheres coated with monoclonal antibodies to human transcobalamin. The cobalamin bound to the holoTC is then released and measured by a competitive binding radioimmunoassay or by fluorescence, depending on the device used.

In 2016, the World Health Organization Committee on Biological Standardization endorsed assigning a holoTC value to the World Health Organization (WHO) 1st International Standard (IS) for vitamin B12 and serum folate, (product number 03/178). The IS for 03/178 and serum samples with different levels of holoTC levels were analyzed in twelve laboratories in eight countries. They concluded to assign holoTC a value of 107 pmol/L to 03/178, corresponding to 0.107 pmol per ampoule for use as the 1st International Standard for vitamin B12, serum folate, and holoTC.\[1\]

REGULATORY STATUS

The Axis-Shield HoloTC RIA is an example of a radioimmunoassay for holoTC that was cleared for marketing by the U.S. Food and Drug Administration (FDA) through the 510(k) process with the following labeled indication for use:

“\[The Axis-Shield HoloTC RIA is an in vitro diagnostic assay for quantitative measurement of the fraction of cobalamin (vitamin B12) bound to the carrier protein transcobalamin in the human serum or plasma. Measurements obtained by this device are used in the diagnosis and treatment of vitamin B12 deficiency.\]

In November 2006, the device Axis-Shield HoloTC Assay, an enzyme immunoassay for holoTC, 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.”

In February of 2013, Active-B12 (Axis-Shield) received FDA approval through the 510(k) process with the following labeled indication for use:

“The Axis-Shield Active-B12 (Holotranscobalamin) assay is an enzyme-immunoassay (EIA) for the quantitative determination of holotranscobalamin (HoloTC) in human serum. HoloTC (vitamin B12 bound to transcobalamin) is used as an aid in the diagnosis and treatment of vitamin B12 deficiency.”

EVIDENCE SUMMARY

Validation of the clinical use of any diagnostic test focuses on three main principles:

1. Analytic validity, the technical feasibility of the test; and
2. Clinical validity, 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. Clinical utility, how the results of the diagnostic test will be used to improve the management of the patient.

ANALYTIC VALIDITY

The technical feasibility of serum holotranscobalamin (holoTC) measurement has been established. As noted in the Description section, serum measurements of holoTC involve the use of standard laboratory immunoassay techniques.

CLINICAL VALIDITY

The diagnostic performance must be compared to the established gold standard for measuring cobalamin deficiency. This is problematic since there is currently no established gold standard to measure cobalamin deficiency. As previously noted, serum levels of total cobalamin have low sensitivity and specificity. In addition there are several reports proposing serum measures of methylmalonic acid (MMA) and homocysteine as an alternative gold standard.[2-5]. It is thought that identification of subclinical disease can prompt early treatment such that clinical symptoms do not develop. Given the absence of a definitive gold standard, confirmation of a diagnosis of subclinical disease is problematic.

Systematic Reviews

In a 2021 systematic review, Wahbeh reported on the role of vitamin B12 and genetic risk factors in the etiology of neural tube defects (NTD).[6] The authors evaluated 40 eligible studies based on specific criterion and found that levels of holotranscobalamin were reported lower in mothers of NTD-affected infants. It was also found that low maternal holotranscobalamin is associated with a strong risk of NTDs in the offspring. Several other maternal factors have also been linked with significant NTD risk in addition to vitamin B12 deficiency including BMI, maternal diet, air pollutants, and low maternal age. The majority of studies on NTDs have a focus on the role of folic acid, therefore a need exists for well-designed studies on the role of risk factors like vitamin B12 deficiency in the etiology of NTDs.
In 2013 Dullemeijer reported on a systematic review and meta-analysis of studies on biomarker responses to B12 supplementation.[7] 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 randomized controlled trials (RCTs) with three holoTC estimates were identified which showed B12 supplementation significantly increased serum or plasma holoTC 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 in 2012 reported on a systematic review of B12 status and its relationship to cognitive decline and dementia.[8] The authors evaluated 35 cohort studies and found serum B12 levels were not associated with cognitive decline or dementia, though four studies found increased risks of cognitive decline or dementia were associated with MMA and/or holoTC levels. Nevertheless, the use of underpowered cohort studies of short duration limits interpretation of these results.

In April 2009, Hoey published a systematic review of the response of various biomarkers to treatment with vitamin B12.[9] Only one RCT utilizing holoTC was identified for the review; therefore the authors concluded that data were insufficient to draw conclusions about the effectiveness of serum holoTC as a biomarker for vitamin B12 status.[10]

Randomized Controlled Trials

Del Bo (2019) reported results of an RCT that assessed the effects of sublingual B12 on serum B12, holotranscobalamin, succinic acid, methionine, a wellness parameter, levels of methylmalonic acid, homocysteine and folate.[11] Individuals with marginal vitamin B12 deficiency (n=40) were randomized to receive low dose (350 μg/week) or high dose (2000 μg/week) sublingual vitamin B12 supplementation. Testing for vitamin B12, related metabolic markers, and blood cell counts was carried out at baseline and after 15, 30, 60, and 90 days from the intervention. No differences were observed between groups. According to a two-way analysis of variance, in both groups, following treatment, statistically significant increases were measured in levels of holotranscobalamin, succinic acid, methionine and wellness parameter (p<0.0001) and statistically significant decreases were measured in levels of methylmalonic acid, homocysteine, and folate, compared with baseline.

In 2015, Dangour published results of an RCT to evaluate the effect of vitamin B-12 supplementation on neurologic and cognitive outcomes in 201 elderly subjects with moderate B-12 deficiency.[12] At 12-month follow-up, B-12 allocation was associated with significant increase of vitamin B-12 concentrations and serum holoTC compared to sham group. However, there were no differences in primary outcome measures of posterior tibial compound muscle action potential amplitude. In addition, there was also no evidence of an effect on any secondary peripheral nerve or central motor function outcome, or on cognitive function or clinical examination.

In 2013, Hill reported on a double-blind, placebo-controlled, randomized study of 100 elderly patients with poor B12 status.[13] 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 holoTC 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 on cognitive functioning in older adults, Eussen measured holoTC 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 holoTC for all biomarkers measured (vitamin B12, MMA, holoTC, homocysteine, and red blood cell folate \([p<0.0002]\)). Specifically for holoTC, 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, holoTC 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 published a smaller trial in 2008. Once again, patients were randomly assigned to cobalamin, cobalamin plus folate, or placebo supplementation in subjects with known mild cobalamin deficiency. Along with serum cobalamin and MMA levels, holoTC was utilized to assess deficiency status and did rise in response to therapy.

**Nonrandomized Studies**

Bondu (2020) assessed the diagnostic accuracy of serum holoTC compared to total B12 and total Homocysteine (HCY) as an indicator of serum B12 status. Participants with B12 deficiency (n=70), borderline B12 deficiency (n=100), and no B12 deficiency (n=47) were assessed for holoTC, HCY, mean corpuscular volume (MCV), folate, hemoglobin and creatinine. There was a strong positive correlation between total Vitamin B12 and holoTC \((r=0.754, \ p\leq 0.001)\) and a strong negative correlation between holoTC and HCY \((r=-0.471, \ p\leq 0.001)\). There was statistically significant concordance between total vitamin B12 and HCY (Kappa index = 0.51, \(p<0.001\)) and between holoTC and HCY (Kappa index = 0.52, \(p\leq 0.001\)). The diagnostic accuracy for detecting a possible or probable clinical B12 deficiency, reported as AUC, for HoloTC, MMA, B12, and Hcy were 0.982 (95% CI 0.979 to 0.984), 0.98 (95% CI 0.978 to 0.983), 0.969 (95% CI 0.966 to 0.972), and 0.898 (95% CI 0.892 to 0.903), respectively. For subclinical B12 deficiency, AUCs were 0.912 (95% CI 0.907 to 0.917) for HoloTC, 0.904 (95% CI 0.898 to 0.909) for MMA, 0.899 (95% CI 0.894 to 0.905) for B12 and 0.789, 95% CI 0.781 to 0.796) for Hcy. HoloTC had significantly greater diagnostic accuracy than B12 \((p=0.02)\) and Hcy \((p<0.001)\), but not MMA \((p=0.15)\).

Jarquin Campos (2020) evaluated the accuracy of four markers for diagnosing B12 deficiency. A population of 11,833 samples were evaluated for B12, HoloTC, MMA, and Hcy. The diagnostic accuracy of each marker for detecting B12 deficiency was determined, with 4cB12 (a combined index of the B12 status) used to define B12 deficiency. The AUC for the detection of subclinical B12 deficiency for HoloTC, MMA, B12, and Hcy was 0.92, 0.91, 0.9, and 0.78, respectively. In women 50 years and older, the AUC for HoloTC (0.93) was significantly higher than for the other measures. For the remainder of the tested population, there were no significant differences in the AUCs of HoloTC, B12, and MMA.

Naik (2018) reported on B12-related biomarkers measured in 110 young, healthy Indian vegetarians. Circulating holoTC, B12, folate, and homocysteine were measured. No participants had clinical signs of B12 deficiency. Receiver operating characteristic curve analysis demonstrated similar area under the curve (AUC) at the B12 concentration of 100 and
150 pmol/l when holoTC (0.777 and 0.784) and total homocysteine (tHcy; 0.924 and 0.928) were used as variables. A cut-off value of 100 pmol/l resulted in the highest sensitivity (77.8%) and specificity (71.05%), with a predictive value of 19.6 pmol/l for holoTC. This cutoff resulted in a sensitivity of 82.72% and specificity of 89.7% with a predictive value of 21.7 µmol/l for homocysteine. Combining B12, holoTC, and tHcy was found to improve diagnostic accuracy.

Greibe (2017) compared the effect of two forms of B12 supplementation (cyano-B12 and hydroxo-B12), taken over eight weeks.[19] Subjects were 51 healthy Indian adults with serum cobalamin measured as under 200pmol/L. They were treated with daily oral supplements of 3-µg cyano-B12 (n = 15), 3-µg hydroxo-B12 (n = 16), or a placebo (n = 20). Baseline and weekly blood samples were analyzed for total cobalamin, holoTC, MMA, and homocysteine. The groups were not significantly different in these measures at baseline. HoloTC, MMA, and homocysteine were not different between groups at any time point. However, total serum cobalamin had a greater increase in the group supplemented with cyano-B12 than the group treated with hydroxo-B12. At eight weeks, the biomarker values were significantly different in the supplemented groups (pooled) than the placebo group.

In 2017, van Wijngaarden reported results of a cross-sectional observational study to investigate associations between B12 intake and biomarkers and among biomarkers.[20] Participants of the B-PROOF study, including 2919 elderly people (65 years of age or older with tHcy levels greater than to equal to 12 µmol/L) were examined for levels of B12, holoTC, MMA, and homocysteine. Authors assessed the association between B12 intake and biomarker status with multivariate regression analysis. They further analyzed the dose-response association between B12 intake and biomarkers and the association of total B12 and holoTC with tHcy and MMA. An association was found between a doubling of B12 intake and increases in total B12 and holoTC (9 and 15%, respectively) and decreases in MMA and tHcy (9 and 2%, respectively). Associations between biomarkers were also reported. Vitamin B12 levels below 330 pmol/L and holoTC levels below 200 pmol/L were associated with a rise in MMA and tHcy. A sharp increase in MMA and tHcy were associated with levels of B12 and HoloTC below 220 and 50 pmol/L respectively.

A 2017 study by Ok Bozkaya examined the relationship between plasma holoTC and serum vitamin B12 in 155 children.[21] Hemoglobin, vitamin B12, folate, ferritin, and holoTC levels were measured and children were divided into two groups based on B12 levels. B12 levels were considered normal above 200pg/mL (n=54) and low less than or equal to 200pg/mL (n=101). The low and normal B12 groups had significantly different mean holoTC levels (21.74 ± 1.14 pmol/L and 44.0 ± 2.7 pmol/L (p<0.01), respectively).

In 2016, van der Zwaluw reported cross-sectional associations between circulating homocysteine, folate, biomarkers of vitamin B12 status and brain volumes.[22] No significant associations were observed for serum vitamin B12 and holoTC. Primary study outcomes from the original randomized, double-blind, placebo-controlled study assessing the efficacy of lowering homocysteine levels by two years of folic acid and vitamin B12 supplementation in the prevention of osteoporotic fractures in elderly people with elevated homocysteine concentration were reported elsewhere.

In a study by Loikas (2003), participants included 226 normal elderly subjects and 80 normal, non-elderly adult Finnish subjects.[23] 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 holoTC results underwent additional testing with MMA; those with
elevated MMA levels were also excluded. In the normal reference population, the holoTC range was 25 to 254 pmol/L with a 95% central reference interval of 37 to 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 holoTC below 37 pmol/L. The frequencies of low holoTC levels increased with increasing pretest probability of cobalamin deficiency. For example, among the sixteen patients thought to have the highest pretest probability of cobalamin deficiency, based on elevated levels of homocysteine and MMA, 100% had low levels of holoTC. 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 potential, possible, or probable cobalamin deficiency. The authors conclude that further studies are needed to confirm the clinical utility and specificity of holoTC in diagnosis of subclinical cobalamin deficiency. Additionally, 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.

Hvas and Nexo (2003) reported on a study of 143 subjects who were divided into four groups, those with a confirmed diagnosis of cobalamin deficiency based on a decreased total serum cobalamin (<200 pmol/L) and increased MMA (>0.70 μmol/L), a second group thought to be normal based on normal values of total serum cobalamin and MMA, and finally two additional groups with an uncertain diagnosis due to conflicting values of total cobalamin and MMA.[24] Although these authors used the reference interval established in the above study (i.e., 24 to 157 pmol/L), the cut-off for a low result was set at 50 pmol/L. Using this cut-off point, measurements of holoTC had a sensitivity of 1.00 and specificity of 0.89 in classifying patients very likely to be, or not be, cobalamin deficient. Among the 73 patients with conflicting levels of MMA and total cobalamin, 39 had low holoTC levels. Without a gold standard, it is difficult to interpret the results in this group with an uncertain diagnosis. As noted by the authors, it is not possible to determine whether or not holoTC correctly classified the individual as deficient or not.

Hermann (2003)[25] reported on another series of patients using the same 37 pmol/L cut-off established by Loikas[23]. This study included 93 omnivorous German controls, and several other groups of patients considered at risk for cobalamin deficiency: 111 German and Dutch vegetarians, 122 apparently health Syrians, 127 elderly Germans, and 92 patients with renal failure. In addition to holoTC, MMA, total serum cobalamin, and homocysteine were measured. A total of 72%, 50%, and 21% of vegetarians, Syrians, and the elderly respectively had holoTC levels of less than 35 pmol/L. Similar to the study above, these low levels of holoTC were associated with either normal or high levels of MMA. Conversely, high levels of MMA were associated with normal holoTC levels in other patients. Again, it is difficult to interpret the clinical significance of these conflicting laboratory values.

Valente (2011) reported on the diagnostic accuracy of holotranscobalamin, MMA, serum cobalamin, and other indicators of tissue vitamin B12 status in an elderly population.[26] Elderly subjects (n=700), age range 63 to 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 to 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 holoTC, 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 AUC showed that serum holoTC 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 serum cobalamin 0.80 (95% CI 0.75 to 0.85), and MMA 0.78 (95% CI 0.72 to 0.83). For these three analytes, the authors constructed a three-zone partition of positive and negative zones and a deliberate indeterminate zone between. The boundaries were values of each test that resulted in a posttest probability of deficiency of 60% and a posttest probability of no deficiency of 98%. The proportion of indeterminate observations for holoTC, cobalamin, and MMA was 14%, 45%, and 50%, respectively.

Heil (2012) evaluated usefulness of holoTC as an initial screening assay for metabolic vitamin B(12) deficiency in a mixed patient population.[27] Three hundred and sixty blood samples were collected by five Dutch hospitals, and vitamin B12 and holoTC 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 holoTC 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 holoTC 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 holoTC did not improve diagnostic accuracy at this cut-off level. The authors concluded that holoTC has a better diagnostic accuracy than vitamin B12 and could replace the existing vitamin B12 assay as a primary screening test in patients suspected of vitamin B12 deficiency. Further randomized, controlled studies are necessary to validate the 32 pmol/L cut-off value established in this study across differing populations. In addition, questions concerning the value of holoTC testing to improve clinical management need to be answered.

Fragasso (2012) conducted a small (n=22) study of serum cobalamin (Cbl) levels in alcoholics who can have falsely increased values of Cbl caused by alcoholic liver disease.[28] A significant positive correlation was found between serum Cbl and holoTC levels however this study is limited by small sample size which restricts conclusions as to the usefulness of holoTC as a measurement for assessing B12 status in alcoholics.

Remacha (2014) evaluated holoTC, MMA and homocysteine levels in 106 patients with low or borderline serum cobalamin (LB12) and in 27 patients with folate deficiency.[29] Although lower levels of holoTC were observed in both LB12 and FOL groups, the concordance between holoTC and MMA was poor. Sobczyrnska-Malefora also found holoTC to be a poor predictor of MMA.[30]

Other recent studies have utilized holoTC as one of a number of measures of cobalamin status.[13, 31-42] 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.

**CLINICAL UTILITY**

Advocates of holoTC testing posit that this laboratory test can identify early subclinical stages of cobalamin deficiency, permitting prompt initiation of treatment, specifically supplementary cobalamin dietary supplementation. This hypothesis was not directly tested in any of the identified published literature. In the absence of an established 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 (e.g., vegetarians, the elderly, 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.

**PRACTICE GUIDELINE SUMMARY**

The measurement of serum holoTC is not specifically addressed in any evidence-based clinical practice guideline.

**SUMMARY**

There is not enough research to know if or how well serum holotranscobalamin (holoTC) testing works as an alternative to total serum cobalamin, levels of methylmalonic acid (MMA), or homocysteine to improve diagnosis and management of vitamin B12 deficiency. No clinical guidelines based on research recommend holoTC testing. Therefore, holoTC testing is considered investigational for all indications.

**REFERENCES**


### CODES

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