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Vitamin B12 Testing in People With Suspected Vitamin B12 Deficiency

Rapid Review

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Abbreviations

AUC	area under the curve
Hcy	homocysteine
HHcy	hyperhomocysteinemia
holoTC	holotranscobalamin
HTA	health technology assessment
MMA	methylmalonic acid

Key Messages

- Findings from 3 diagnostic accuracy studies indicate that individual tests are insufficient to diagnose vitamin B12 deficiency, and a testing strategy that uses homocysteine and methylmalonic acid should be used in individuals suspected or at risk of vitamin B12 deficiency. This is supported by a health technology assessment that concluded that the current evidence does not provide enough information to determine the most appropriate test, or combination of tests to use in these patients.
- The patient populations varied in the primary studies included in the systematic reviews and in the primary studies identified in this review, which may impact the generalizability of the results.
- Reference standards and cut-off values (i.e., thresholds) used to diagnose vitamin B12 deficiency varied in the primary studies. Presentation of units (e.g., pg/mL, pmol/L) also varied, making it difficult to compare results across studies.
- No studies were identified that evaluated the clinical utility of vitamin B12 testing in people with suspected vitamin B12 deficiency.
- No studies were identified that evaluated the cost-effectiveness of vitamin B12 testing in people with suspected vitamin B12 deficiency.
- No evidence-based guidelines were identified regarding the use of vitamin B12 testing in people with suspected vitamin B12 deficiency.

Context and Policy Issues

Vitamin B12, also called cobalamin, is a water-soluble B vitamin found in foods derived from animal products and from fortified cereals.[1,2] The prevalence of vitamin B12 deficiency in the general population differs between older and younger people (e.g., in the UK 6% of those < 60 and 20% of those ≥ 60), and is also dependent on geographical location (e.g., 6% in the US, 40% in Latin American countries, 70% in East India).[2] Most cases of vitamin B12 deficiency (in resource-rich settings) are due to malabsorption. This may be caused by pernicious anemia, an autoimmune condition in which antibodies to intrinsic factor are produced, which results in the inability for vitamin B12 to be absorbed. Other reasons for malabsorption may be from underlying diseases, such as Crohn disease and celiac disease, interaction with medication (e.g., proton pump inhibitor, metformin), and in those exposed to nitrous oxide (i.e., used for anesthesia or recreationally).[1-3] Vitamin B12 deficiency may also be caused by diet (e.g., vegan diet, breastfed neonates born to individuals who are B12 deficient).[1,4]

As vitamin B12 is stored in body tissue (mainly the liver) subclinical deficiency develops over the course of several years (e.g., 5 to 10 years).[1] Manifestations of vitamin B12 deficiency may be mild, such as fatigue and palpitations, but may progress to neurologic manifestations, such as peripheral neuropathy and dementia-like disease.[3]

There is currently no agreed-upon reference standard for measuring B12, and all are susceptible to confounding factors. A lack of agreement around cut-off levels (i.e., thresholds) to diagnose deficiency adds another layer of difficulty in diagnosing deficiency, as these thresholds may differ between guidelines, intuitions, and laboratories.

The 4 commonly used tests for vitamin B12 deficiency are briefly described in the following:

- Serum vitamin B12 is typically the standard initial diagnostic test.[1] It includes the total level of B12 in the blood, made up of active B12 (i.e., holotranscobalamin [holoTC]), which is available to be used by the body, and inactive B12 (i.e., holohaptocorrin), which cannot be used by the body.[4] Deficiency typically presents in the serum once there is severe clinical deficiency.[3] There is no agreed-upon total serum B12 level to diagnose deficiency, although lower than 148 pmol/L (200 ng/L) is often used.[1,4]
- HoloTC measures the level of active (i.e., biologically available) B12 in the blood,[1] and represents approximately 20% of the B12 measured in blood serum.[5] The values for holoTC in healthy individuals are 35 pmol/L to 171 pmol/L, although lower and upper reference intervals vary greatly.[4]
- Homocysteine (Hcy) levels may accumulate in the blood early in the course of vitamin B12 deficiency;[4] however, elevated levels of Hcy might also be found in people with folate deficiency and those with some diseases (e.g., renal failure).[1,4] There is no consensus on the reference range, although most laboratories use higher than 15 µmol/L as the level to define hyperhomocysteinemia (HHcy).[4]
- Methylmalonic acid (MMA) can be measured in the blood or urine and raises with vitamin B12 deficiency.[1] Although MMA may be falsely elevated in people with renal disease[1] and small bowel bacterial overgrowth, high levels of MMA (e.g., > 0.75 µmol/L) are a strong marker of vitamin B12 deficiency.[4] MMA is a high-cost test, which limits its widespread use.[4]

Current treatment for vitamin B12 deficiency includes supplements, which can be administered orally, sublingually, and parenterally (e.g., intramuscular injection). Route of administration may differ depending on the cause of deficiency. There are few safety concerns around vitamin B12 supplementation, as when stores of vitamin B12 are adequate, excess vitamin B12 is excreted.[6]

Routine evaluation for vitamin B12 deficiency in the general population was reviewed in a 2015 CADTH report.[7] However, testing in populations suspected to be vitamin B12 deficient (e.g., people with the presence of unexplained neurologic abnormalities, people with vegan diets, neonates born to individuals who are vitamin B12 deficient, people taking metformin) may differ.

The objective of this report is to summarize the evidence regarding the diagnostic test accuracy, the clinical utility, the cost-effectiveness, and the recommendations from evidence-based guidelines of vitamin B12 testing in people with suspected vitamin B12 deficiency.

Research Questions

1. What is the diagnostic accuracy of vitamin B12 testing for the diagnosis of vitamin B12 deficiency in people with suspected vitamin B12 deficiency?
2. What is the clinical utility of vitamin B12 testing in people with suspected vitamin B12 deficiency?
3. What is the cost-effectiveness of vitamin B12 testing in people with suspected vitamin B12 deficiency?

4. What are the evidence-based guidelines regarding the use of vitamin B12 testing in people with suspected vitamin B12 deficiency?

Methods

Literature Search Methods

A limited literature search was conducted by an information specialist on key resources including MEDLINE, the Cochrane Database of Systematic Reviews, the international health technology assessment (HTA) database, the websites of Canadian and major international health technology agencies, as well as a focused internet search. The search strategy comprised both controlled vocabulary, such as the National Library of Medicine's MeSH (Medical Subject Headings), and keywords. The main search concepts were Vitamin B12 deficiency and testing. A second search was conducted by applying the diagnostic test accuracy filter to the Vitamin B12 deficiency concept. Comments, newspaper articles, editorials, and letters were excluded. Where possible, retrieval was limited to the human population. The search was also limited to English-language documents published between January 1, 2012, and January 28, 2022.

Selection Criteria and Methods

One reviewer screened citations and selected studies. In the first level of screening, titles and abstracts were reviewed and potentially relevant articles were retrieved and assessed for inclusion. The final selection of full-text articles was based on the inclusion criteria presented in Table 1.

Exclusion Criteria

Articles were excluded if they did not meet the selection criteria outlined in Table 1, if they were duplicate publications, or if they were published before 2012. Systematic reviews in which all relevant studies were captured in other more recent or more comprehensive systematic reviews were excluded. Primary studies retrieved by the search were excluded if they were captured in 1 or more included systematic reviews. Guidelines with unclear methodologies were also excluded.

Critical Appraisal of Individual Studies

The included publications were critically appraised by 1 reviewer using the following tools as a guide: A MeaSurement Tool to Assess Systematic Reviews 2 (AMSTAR 2)[8] for systematic reviews and the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) checklist[9] for diagnostic test accuracy studies. Summary scores were not calculated for the included studies; rather, the strengths and limitations of each included publication were described narratively.

Summary of Evidence

Quantity of Research Available

A total of 272 citations were identified in the literature search. Following screening of titles and abstracts, 245 citations were excluded and 27 potentially relevant reports from the electronic search were retrieved for full-text review. Six potentially relevant publications were retrieved from the grey literature search for full-text review. Of these potentially relevant articles, 27 publications were excluded for various reasons, and 6 publications met the inclusion criteria and were included in this report. These comprised 1 HTA and 5 diagnostic test accuracy studies. Appendix 1 presents the PRISMA[10] flow chart of the study selection.

Additional references of potential interest are provided in Appendix 5.

Summary of Study Characteristics

One HTA[11] and 5 diagnostic test accuracy studies[12-16] were identified for inclusion in this review. No relevant systematic reviews, randomized controlled trials, economic evaluation studies, or evidence-based guidelines were identified.

Additional details regarding the characteristics of the included publications are provided in Appendix 2.

Study Design

One health technology report[11] included 4 systematic reviews to answer 4 research questions. Two of these research questions were relevant to the present review, and the systematic

Table 1: Selection Criteria

Criteria	Description
Population	People of all ages with suspected vitamin B12 deficiency (e.g., presence of unexplained hematologic or neurologic abnormalities [e.g., anemia, macrocytosis, peripheral neuropathy, dementia], or due to variables such as age, diet, family and medical history, or lifestyle)
Intervention	Vitamin B12 testing using complete blood count, blood film, serum tests, or holotranscobalamin testing
Comparator	Q1: No comparator Q2 to Q3: No testing for vitamin B12 deficiency Q4: Not applicable
Reference standard	Q1: Any reference standard Q2 to Q4: Not applicable
Outcomes	Q1: Diagnostic accuracy (e.g., sensitivity, specificity, positive predictive value, negative predictive value) Q2: Clinical utility (e.g., time to treatment, severity of symptoms [e.g., fatigue, mouth pain, numbness, weakness], quality of life, adverse events [e.g., irreversible neuropathy]) Q3: Cost-effectiveness (e.g., cost per quality-adjusted life-year gained) Q4: Recommendations regarding best practices (e.g., appropriate patient populations, recommended testing strategies)
Study designs	Health technology assessments, systematic reviews, randomized controlled trials, non-randomized studies, economic evaluations, evidence-based guidelines

reviews to answer these research questions had broader inclusion criteria than the present review. Specifically, some of the included studies for the diagnostic test accuracy research question (research question 2), included primary studies that were not specific to people suspected of vitamin B12 deficiency. For example, 48% (26 out of 54) of the included studies in 1 systematic review had patients with no defined indications. Only the characteristics and results of the subset of relevant studies will be described in this report. Within the HTA report,[11] 1 systematic review did not identify any included studies and the other systematic reviews included 4 primary studies that were relevant to the current report and published between 2011 to 2015.

Five diagnostic test accuracy studies[12-16] evaluated suspected vitamin B12 deficiency from participants diagnosed with existing conditions from various clinics, and included both outpatients and inpatients. One diagnostic test accuracy study, by Warendorf et al. (2021),[12] used a respective cohort study design, a single-gate approach for patient selection, and a single set of eligibility criteria. Data were retrieved from first-referral outpatient records among those who had been diagnosed with polyneuropathy between January 1, 2013, and January 1, 2018, and whose records had laboratory test results for vitamin B12, MMA, and Hcy. Four diagnostic test accuracy studies used a cross-sectional design, a single-gate approach for patient selection, and a single set of eligibility criteria. Ao et al. (2017)[13] included patients with Crohn disease attending the gastroenterology clinic at a university hospital. No date were provided for patient recruitment. Vashi et al. (2016)[14] included a consecutive series of patients with cancer first seen at the Cancer Treatment Centers of America at the Midwestern Regional Medical Center between April 2014 and June 2014. Beletić et al. (2015) included patients with chronic obstructive pulmonary disease at the Clinic for Lung Disease and the Center for Medical Biochemistry of the Clinical Center of Serbia. Among the 50 patients included, 15 were attending the outpatient department of the clinic and 35 were hospitalized and enrolled upon remission. No date was provided for patient recruitment.[15] Finally, Cinemre et al. (2015) included patients in the hematology department with a diagnosis of myeloproliferative disease. No date was provided for patient recruitment.[16]

Country of Origin

The HTA was conducted by the Swiss Federation and, as such, the applicability of the findings have been discussed within the Swiss context.[11]

The diagnostic test accuracy studies were conducted in, and enrolled patients from, Japan,[13] the Netherlands,[12] Serbia,[15] Turkey,[16] and the US.[14]

Patient Population

The HTA report[11] included 2 systematic reviews relevant to this review that included patients with a clinical suspicion of vitamin B12 deficiency (e.g., those with macrocytosis, dementia, paresthesia, polyneuropathy, glossitis, malnutrition) and patients in high-risk populations (e.g., those who are post-bariatric surgery or gastric resection, vegans, vegetarians, age ≥ 65 years). In the other systematic review, among the 4 studies that are relevant to this review, the number of participants ranged from 77 to 1,279. The age of the participants was reported in 3 of these 4 studies, with mean ages of 55.09, 59, and 81. The setting in which the patients were identified from was not well reported, but included a natural medicine-oriented doctor's office.

The diagnostic test accuracy study by Warendorf et al. (2021) included 311 first-referral outpatients diagnosed with polyneuropathy (mean age of 63.0 years).[12] The study by Ao et al.

(2017) included 48 outpatients of a gastroenterology clinic who had Crohn disease (mean age of 40.1 years).[13] Vashi et al. (2016) included 316 patients with cancer from a Cancer Treatment Centres of America who were older than 18 (mean age of 52.5 years).[14] Beletić et al. (2015) included 50 people from a clinic for lung diseases and a centre for medical biochemistry who had chronic obstructive pulmonary disease, diagnosed according to the Global Initiative for Chronic Obstructive Lung Disease (mean age of 49.0 years).[15] Finally, Cinemre et al. (2015) included 58 patients followed in a hematology department who had myeloproliferative disorders (mean age of 61 years).[16]

Interventions and Comparators

The 2 systematic reviews in the HTA[11] that are relevant to this review compared diagnostic serum testing (i.e., B12, holoTC, MMA) to no testing for the review on clinical effectiveness, and any diagnostic serum screening or test (i.e., total serum B12, holoTC, MMA) as the index test compared to any of these as the reference test. The 4 relevant primary studies extracted from this review evaluated the diagnostic accuracy of vitamin B12 with holoTC as the reference test, vitamin B12 with MMA as the reference test, and MMA with holoTC as the reference test.

The diagnostic test accuracy studies evaluated several index and reference tests. Warendorf et al. (2021) evaluated total vitamin B12 using 3 different reference tests (MMA, Hcy, and Hcy and/or MMA).[12] Ao et al. (2017) evaluated serum concentrations of vitamin B12 with plasma concentration of Hcy as the reference test.[13] Vashi et al. (2016) evaluated vitamin B12 with the Fedosov wellness quotient as the reference test. (The Fedosov wellness quotient uses a combined vitamin B12, MMA, and Hcy approach.)[14] Beletić et al. (2015) used 3 different thresholds of Hcy as the index test and 2 different vitamin B12 thresholds as the reference tests.[15] Cinemre et al. (2015) evaluated serum vitamin B12, holoTC, and total plasma as the index tests against serum MMA as the reference test.[16] As the studies were a retrospective cohort and 4 cross-sectional designs, there were no follow-up periods.

In all of the diagnostic test accuracy studies, all participants received the same index and reference tests. For most studies, it was not clearly stated if there was an interval between tests; however, as they were cross-sectional, it is likely that testing occurred at the same time.[13-16] As Warendorf et al. (2021) was a retrospective cohort study that used patient record, it was stated that the tests were done within 60 days of each other.[12] In 4 of the diagnostic test accuracy studies,[12,13,15,16] it was unclear if the results of the index tests were read without the knowledge of the results of the reference tests. Vashi et al. (2016) stated that the results of all tests were determined simultaneously in each patient.[14]

Outcomes

In the HTA report,[11] the systematic review on clinical effectiveness aimed to report on safety (e.g., adverse events from testing), treatment (e.g., oral supplements), and effectiveness (e.g., improvement of symptoms) outcomes. The systematic review on the diagnostic accuracy of vitamin B12 testing reported on several statistical measures of diagnostic test accuracy, including sensitivity, specificity, and positive and negative predictive values.

The diagnostic test accuracy studies calculated various parameters of diagnostic performance. All 5 studies reported the area under the curve (AUC).[12-16] Warendorf et al. (2021)[12] also reported the threshold vitamin B12 levels to obtain 90% and 95% sensitivity. Vashi et al. (2016)[14] reported the sensitivity and specificity. Cinemre et al. (2015)[16] also

reported the sensitivity, specificity, likelihood ratio positive, likelihood ratio negative, positive predictive value, and negative predictive value.

Summary of Critical Appraisal

Evidence Reviews

The systematic reviews in the HTA report[11] were well-conducted and reported. The review methods were defined before the conduct of the reviews and a protocol was written and peer-reviewed by independent anonymous reviewers. The Participants, Intervention, Comparator, Outcomes (PICO) criteria were clearly defined for each of the systematic reviews that were undertaken. Several electronic databases were searched, reducing the risk of study selection bias, and the searches were provided, allowing for reproducibility. Identification of the included studies and risk of bias were performed by 2 independent reviews. The 1 minor limitation was around data extraction, which was performed by 1 reviewer and verified by a second reviewer.

Diagnostic Test Accuracy Studies

There were several common strengths in the 5 diagnostic test accuracy studies:[12-16] the objectives, index tests, reference tests, and main outcomes were clearly described; all patients received the same index and reference tests; the thresholds used for the screening test were prespecified and supported by literature or by the manufacturers' instructions; most studies included all participants in the analysis and among those that did not, the reasons for exclusion were provided and were clinically relevant; all studies avoided a case-control study design; and all studies disclosed the source of funding. One study described the method of patient selection.[14] One study reported that the results of the screening tests were read without knowledge of the other tests.[14] Four studies disclosed that their authors had no conflicts of interest.[12-15] Although all studies included patients who were suspected of vitamin B12 deficiency, due to their underlying health conditions (e.g., cancer, myeloproliferative disease, Crohn disease, chronic obstructive pulmonary disease), the results from the individuals studies may not be generalizable to all patients who are suspected of vitamin B12 deficiency.

Additional details regarding the strengths and limitations of the included publications are provided in Appendix 3.

Summary of Findings

Appendix 4 presents the main study findings and authors' conclusions. The data from the 4 relevant studies included in the systematic review of the HTA[11] are reported in the Diagnostic Parameters section. Fourteen different index and reference tests were evaluated.

Diagnostic Accuracy of Vitamin B12 Testing

Area Under the Curve

Vitamin B12 Versus Homocysteine

Two diagnostic test accuracy studies reported the AUC for different thresholds of vitamin B12 with Hcy (> 14 µmol/L) as the reference test. Warendorf et al. (2021)[12] reported an AUC of 0.61. Ao et al. (2017)[13] reported an AUC of 0.73, which was statistically significant (95% confidence interval, 0.606 to 0.900), with the adequate cut-off value of 503 pg/mL for vitamin B12.

Vitamin B12 Versus Methylmalonic Acid

One diagnostic test accuracy study, by Warendorf et al. (2021),[12] reported the AUC for vitamin B12 with MMA (> 0.29 µmol/L) as the reference test. This was the highest AUC (0.72) in the study, which also evaluated Hcy and MMA and/or Hcy as the reference tests.

Vitamin B12 Versus Methylmalonic Acid and/or Homocysteine

One diagnostic test accuracy study, by Warendorf et al. (2021),[12] reported an AUC of 0.65 for vitamin B12 with MMA (> 0.29 µmol/L) and/or Hcy (> 14 µmol/L) as the reference test.

Vitamin B12, Homocysteine, Methylmalonic Acid Versus Fedosov Wellness Quotient (Combined Vitamin B12, Methylmalonic Acid, Homocysteine)

One diagnostic test accuracy study, by Vashi et al. (2016),[14] reported the AUC for 3 index tests: vitamin B12 (< 300 pM/mL), Hcy (> 12 µmol/L), and MMA (> 260 nmol/L) with the combined vitamin B12, MMA, and Hcy as the reference test. MMA performed best with an AUC of 0.98. AUC for vitamin B12 and Hcy were similar, at 0.83 and 0.85, respectively.

Homocysteine Versus Vitamin B12

One diagnostic test accuracy study, by Beletić et al. (2015),[15] reported the AUC for Hcy at 3 different thresholds (10 µmol/L, 12 µmol/L, and 15 µmol/L) with vitamin B12 at thresholds (203 ng/L and 473 ng/L) as the reference test. AUCs ranged from 0.504 to 0.842, with a Hcy of 15 µmol/L and a vitamin B12 of 203 ng/L having the highest AUC (0.842). However, none of the values were considered statistically significant of Hcy as a predictor of vitamin B12 deficiency.

Holotranscobalamin, Homocysteine Versus Methylmalonic Acid

One diagnostic test accuracy study, by Cinemre et al. (2015),[16] reported the AUC for 2 index tests, holoTC (< 35 pmol/L) and Hcy (> 13 mol/L), with an age-adjusted MMA as the reference test. Both holoTC (AUC = 0.822; 95% confidence interval, 0.70 to 0.91; P ≤ 0.001) and Hcy (AUC = 0.662; 95% confidence interval, 0.53 to 0.78; P = 0.03) were found to be statistically significant.

Diagnostic Parameters

Vitamin B12 Versus Holotranscobalamin

Three diagnostic test accuracy studies reported the sensitivity and specificity for vitamin B12 with holoTC as the reference test. Schwarz et al. (2015), a primary study included in the HTA,[11] evaluated the diagnostic accuracy of vitamin B12 using 2 different methods, chemiluminescent immunoassay (threshold of < 156 pmol/L) and microbiological tests with microtitre (threshold of < 212 pmol/L), with holoTC at 2 thresholds as the reference test. A holoTC threshold of less than 50 pmol/L was considered subclinical or possibly deficiency, and less than 35 pmol/L was considered probably deficient. Among these, the vitamin B12 microbiological tests with microtitre and holoTC (< 35 pmol/L) had the highest sensitivity at 71% (specificity = 95%). Sensitivity for other test comparisons ranged from 38% to 53% and specificity ranged from 90% to 96%.

Heil et al. (2012), included in the HTA,[11] reported a sensitivity of 64% and a specificity of 88% for vitamin B12 (< 146 pmol/L) with holoTC (< 21 pmol/L). Schrempf et al. (2011) reported a lower sensitivity (56.3%), but at higher thresholds for both vitamin B12 (< 206 pmol/L) and holoTC (< 42 pmol/L).

Vitamin B12 Versus Homocysteine

Warendorf et al. (2021)[12] reported that the best trade-off was at a vitamin B12 threshold of 257 pmol/L and a Hcy of greater than 14 µmol/L (sensitivity = 79%; specificity = 44%). At the higher vitamin B12 threshold of 374 pmol/L, sensitivity increased to 97%, but the specificity lowered to 10%.

Vitamin B12 Versus Methylmalonic Acid

Three diagnostic test accuracy studies evaluated different thresholds of vitamin B12 with MMA as the reference test. Heil et al. (2012), included in the HTA,[11] reported a sensitivity of 53% and a specificity of 81% for vitamin B12 (< 146 pmol/L) with MMA (> 0.45 µmol/L) as the reference test. Both Schrempf et al. (2011) and Warendorf et al. (2021)[12] reported higher sensitivity, but lower specificity than Heil et al. (Schrempf: sensitivity = 66.2%, specificity = 62.1%; Warendorf: sensitivity = 69%, specificity = 66%) using a higher vitamin B12 (Schrempf: < 206 pmol/L; Warendorf: < 213 pmol/L) and a lower MMA (Schrempf: > 0.298 µmol/L; Warendorf: > 0.29 µmol/L) threshold.

Vitamin B12 Versus Methylmalonic Acid and/or Homocysteine

Warendorf et al. (2021)[12] evaluated vitamin B12 with the reference test of MMA (> 29 µmol/L) and/or Hcy (> 14 µmol/L). Results were similar between the reference test and using only Hcy (> 14 µmol/L).

Vitamin B12, Homocysteine, Methylmalonic Acid Versus Fedosov Wellness Quotient (Combined Vitamin B12, Methylmalonic Acid, Homocysteine)

One diagnostic test accuracy study, by Vashi et al. (2016),[14] evaluated 3 index tests, vitamin B12, Hcy, and MMA, with a combined reference test that included vitamin B12, MMA, and Hcy. MMA (> 413.5 nmol/L) had the highest sensitivity and specificity, at 86% and 99%, respectively. Vitamin B12 (< 385 pg/mL) (86% and 80%, respectively) and Hcy (> 15.5 µmol/L) (71% and 95%, respectively) were less sensitive and specific.

Holotranscobalamin, Homocysteine Versus Methylmalonic Acid

One diagnostic test accuracy study, by Cinemre et al. (2015),[16] reported that holoTC (≤ 40.6 pmol/L) had a greater sensitivity, specificity, positive likelihood ratio, positive predictive value, and negative predictive value than Hcy (> 14 mol/L), with age-adjusted MMA values as the reference test. HoloTC had a sensitivity of 75% and a specificity of 78%.

Methylmalonic Acid Versus Holotranscobalamin

Schwarz et al. (2015), a primary study in the HTA,[11] evaluated the diagnostic accuracy of MMA (> 0.30 µmol/L) with holoTC at 2 thresholds as the reference test. A threshold of less than 50 pmol/L was considered subclinical or possibly deficiency, and less than 35 pmol/L was considered probably deficient. MMA was not sensitive at either holoTC threshold (41% and 40%).

Vitamin B12, Holotranscobalamin, Methylmalonic Acid Versus Red Cell Cobalamin

Valente et al. (2011), a primary study in the HTA report,[11] evaluated the diagnostic accuracy of 3 different index tests, vitamin B12, holoTC, and MMA with red cell cobalamin (< 33 pmol/L) as the reference test. MMA (> 0.36 µmol/L) had the highest sensitivity, but the lowest specificity, at 81% and 63%, respectively.

Clinical Utility of Vitamin B12 Testing

No relevant evidence regarding the comparative clinical utility of vitamin B12 testing was identified; therefore, no summary can be provided.

Cost-effectiveness of Vitamin B12 Testing

No relevant evidence regarding the cost-effectiveness of vitamin B12 testing was identified; therefore, no summary can be provided.

Guidelines

No relevant evidence-based guidelines on vitamin B12 testing were identified; therefore, no summary can be provided.

Limitations

There were some limitations to the studies that would prevent a definitive conclusion on the diagnostic accuracy, clinical utility, and cost-effectiveness of vitamin B12 testing in those suspected of having vitamin B12 deficiency.

The systematic reviews conducted in the HTA included broader populations than were relevant for this review, particularly around the populations included in the primary studies. The HTA[11] included studies that were conducted in WHO mortality stratum A countries, which may have omitted relevant studies. A common theme in the primary studies included in the systematic review was a lack of clear reporting around the clinical characteristics of the populations, which makes it difficult to determine if they are "suspected" of vitamin B12 deficiency. The primary studies that were identified as being relevant to this review had several different index and reference tests, and used different thresholds to define vitamin B12 deficiency.

Similar to the primary studies identified in the HTA, the diagnostic test accuracy studies[12-16] used different index and reference tests, as well as different thresholds to define vitamin B12 deficiency. As this review included people with suspected vitamin B12 deficiency, the primary studies included people with underlying health conditions (e.g., cancer, myeloproliferative disorders, Crohn disease). These diseases may impact the health of these individuals differently; therefore, results in the studies may not be generalizable to all people suspected of vitamin B12 deficiency. Additionally, 3 of the 5 primary studies[13,15,16] included fewer than 60 participants, which may not be considered a sufficient sample size.[17] Several subpopulations were not identified in the included studies (for example, pregnant individuals suspected of vitamin B12 deficiency, babies born to individuals who are vitamin B12 deficient, and people with a vegan diet suspected of vitamin B12 deficiency).

No studies on the clinical utility or cost-effectiveness of vitamin B12 testing were identified. It is unclear which test(s) (e.g., serum vitamin B12, holoTC, Hcy, MMA) may result in improved clinical outcomes for patients who are suspected of having vitamin B12 deficiency and which, if any, would be cost-effective.

No evidence-based guidelines were included. One potential guideline was identified, but was excluded due to a lack of description of the methods to identify the included studies. This guideline has been listed in Appendix 5.

None of the included primary studies were conducted in Canada, and the HTA[11] was not conducted or intended for professionals in Canada. Therefore, the generalizability of the findings from the included literature to the Canadian setting are unclear.

Conclusions and Implications for Decision- or Policy-Making

This review comprised 1 HTA,[11] which included 4 relevant diagnostic test accuracy studies, and 5 diagnostic test accuracy studies.[12-16] No relevant evidence was identified regarding the clinical utility or cost-effectiveness of vitamin B12 testing in people with suspected vitamin B12 deficiency, and no evidence-based guidelines regarding the use of vitamin B12 testing in people with suspected vitamin B12 deficiency were identified.

The results from this review are similar to the recently published HTA by the Swiss Federation.[11] Although covering a similar publication period, the Swiss HTA only included studies from countries listed under the WHO mortality stratum A. This would have excluded 2 of the studies included in this review, which were conducted in Serbia[15] and Turkey.[16] Warendorf et al. (2021)[12] would not have been captured by the search (November 4, 2020). As a list of excluded studies and the reason for exclusion was not provided, it is unclear why Ao et al. (2017)[13] and Vashi et al. (2016)[14] were not included in their review.

Evidence from 3 of the 5 diagnostic test accuracy studies[12,14,15] identified by our search (i.e., not those in the HTA systematic review) reported that a single test is insufficient to diagnose vitamin B12 deficiency. Two of these studies[12,14] concluded that Hcy and MMA should be used in this population in addition to serum B12. This was supported by the included HTA,[11] published in 2021, which concluded that regardless of the results of the first-line test (i.e., total vitamin B12 or holoTC), these results needed to be supported with second-line testing to have sensitive and specific test results. The other 2 diagnostic test accuracy studies did not report on vitamin B12 as an index test, so could not contribute to this conclusion.[13,16] Some studies did report statistically significant results. For example, 1 study, with 58 patients with myeloproliferative disorders, reported that holoTC was more sensitive and specific, had a greater positive likelihood ratio, and greater positive and negative predictive values than Hcy to diagnose vitamin B12 deficiency. However, caution must be taken when considering this result, as this was among a small group of patients with a rare disease, which may limit generalizability.[16]

Fourteen different index test and reference test comparisons were reported in the studies in this report. Vitamin B12, holoTC, Hcy, and MMA were all evaluated as both index and reference tests. Other reference tests included MMA and/or Hcy, the Fedosov wellness quotient (combined vitamin B12, Hcy, and MMA), and red cell cobalamin.

Vitamin B12 versus HoloTC: Three diagnostic test accuracy studies, all included in the systematic review in the HTA,[11] reported sensitivities ranging from 38% to 71%, and specificities ranging from 50.5% to 96%, all using different thresholds for both vitamin B12 and holoTC.

Vitamin B12 versus Hcy: One study, in patients with Crohn disease, reported that serum vitamin B12 is a determinant for hyperhomocysteinemia, at a serum vitamin B12 threshold

of 503 pg/mL.[13] At a similar vitamin B12 threshold (374 pmol/L), 1 study in patients with polyneuropathy reported high sensitivity (97%), but low specificity (10%).[12]

Vitamin B12 versus MMA: Three diagnostic test accuracy studies, 2 of which were included in the systematic review in the HTA, varied in sensitivity and specificity, and the thresholds used.[11,12] Two of these studies used similar thresholds for both tests and reported similar sensitivities (66.2% and 69%) and specificities (62.1% and 66%).

Vitamin B12 versus MMA and/or Hcy: One study used MMA and/or Hcy as the reference test and found similar results for AUC, sensitivity, and specificity as when Hcy was used as the reference test alone.[12]

Vitamin B12, Hcy, MMA versus Fedosov wellness quotient (combined vitamin B12, MMA, Hcy): One study, in patients with cancer, reported that MMA had the highest AUC (0.98) with the combined reference test compared to the index tests of vitamin B12 and Hcy.[14]

Hcy versus vitamin B12: Hcy evaluated at 3 thresholds, in patients with chronic obstructive pulmonary disease, was not found to be a good predictor of vitamin B12 deficiency with vitamin B12 as the reference test.[15]

HoloTC, Hcy versus MMA: One study, in patients with myeloproliferative disorders, reported that holoTC and Hcy were both statistically significant when compared to age-adjusted MMA, and that holoTC had greater sensitivity and specificity than Hcy.[16]

MMA versus HoloTC: One study, included in the systematic review in the HTA,[11] reported that MMA was not sensitive at either of 2 different holoTC thresholds.

Vitamin B12, HoloTC, MMA versus red cell cobalamin: One study, included in the systematic review in the HTA,[11] evaluated 3 different index tests with red cell cobalamin as the reference standard. MMA had the highest sensitivity among the 3 index tests, but the lowest specificity.

The limitations of the included literature should be considered when interpreting the findings of this report. The systematic review in the HTA[11] was well-conducted and well reported, but the primary studies included were rated as having very low or low certainty of evidence, which impacts the confidence in the reported effect estimates. All included primary studies had clear objectives, well described tests and thresholds to define vitamin B12 deficiency, avoided a case-control design, and reported the source of funding, but they also had limitations that impact our confidence in the results. Four[12,13,15,16] of 5 studies did not report on the method of patient selection, or whether the results of the index test were read without knowledge of the results of the reference standard. These limitations may result in bias in the selection of patients and in determining the results of the tests.

As levels of subclinical and clinical vitamin B12 deficiency varies in different countries, further research investigating the diagnostic accuracy of serum vitamin B12, holoTC, Hcy, and MMA in the Canadian setting would provide additional information on what tests should be used in this population. Additional studies in other people at risk should also be conducted (e.g., people with vegan diets). Clinical utility research is needed to evaluate the benefits and harms of testing in patients suspected of vitamin B12 deficiency. Cost-effectiveness studies in the Canadian health care context should also be undertaken, as the Canadian health care system and the disperse geographical landscape could contribute to the cost-effectiveness. The Hcy plasma sample must be kept cool and then centrifuged and removed from the red cells within 2 hours of collection, and MMA uses gas chromatography–mass spectrometry,

which currently has a high cost;[4] it is unknown if these factors would influence the cost-effectiveness of including Hcy and MMA in this population.

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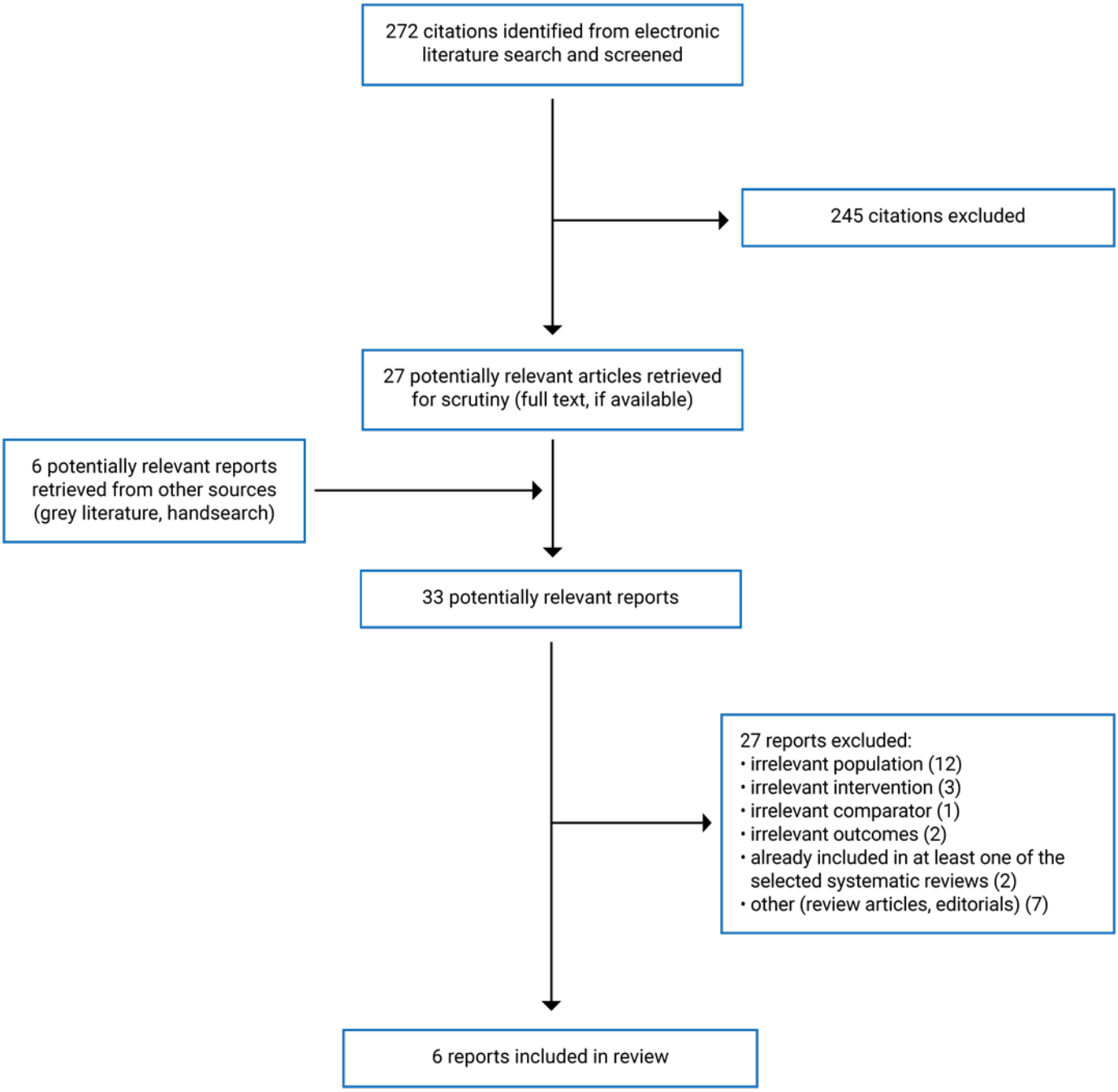
Appendix 1: Selection of Included Studies

Figure 1: Selection of Included Studies



Appendix 2: Characteristics of Included Publications

Note that this appendix has not been copy-edited.

Table 2: Characteristics of Included Health Technology Assessment

Study citation, country, funding source	Study designs and numbers of primary studies included	Population characteristics	Intervention and comparator(s)	Clinical outcomes, length of follow-up
Anastasi et al. (2021)[11] Switzerland **Funding**: NR	**RQ1 study design**: Systematic reviews, randomized, prospective non-randomized trials) **Number of primary studies**: 0 studies were identified **RQ2 study design**: SRs of diagnostic accuracy studies, if no SRs, primary diagnostic test accuracy studies will be considered **Number of primary studies:** One SR (54 primary) studies, plus 6 additional primary studies **Number of relevant primary studies**: 4 primary studies (based on information provided)	Patients with a clinical suspicion of, or at high-risk for, vitamin B12 deficiency Relevant population: patients with a clinical suspicion of vitamin B12 deficiency	**Q1 Interventions**: Diagnostic serum testing (total serum B12, holoTC, MMA) **Q1 Comparator**: No testing **Q2 Index tests**: Any diagnostic screening/ test of vitamin B12 (total serum B12, holoTC, MMA) **Q2 Reference tests**: Any diagnostic screening/test of vitamin B12 (total serum B12, holoTC, MMA)	**RQ1 outcomes**: Safety [adverse events from testing (blood draw) or treatment (oral, nasal or intramuscular)]; Effectiveness [Improvement of symptoms, signs, or quality of life within a defined period (e.g. anaemia, neurological symptoms) and acceptable serum vitamin B12 levels established, or prevention of deficiency development within a defined period] **RQ2 outcomes**: Diagnostic test accuracy (sensitivity, specificity, positive/negative predictive values, false positives, false negatives, invalid/uninterpretable results, positive predictive value, negative predictive, value confirmed by a valid reference test)

holoTC = holotranscobalamin; MMA = methylmalonic acid; NR = not reported; NA = not applicable; RQ = Research Question; SR = systematic review.

Table 3: Characteristics of Included Primary Clinical Studies

Study citation, country, funding source	Study design	Population characteristics	Intervention and comparator(s)	Clinical outcomes, length of follow-up
Warendorf et al. (2021)[12] The Netherlands **Funding**: No funding	Retrospective cohort study	**Inclusion criteria**: First-referral outpatients diagnosed with polyneuropathy **Exclusion criteria**: folate deficiency (<6.8 µmol/L), vitamin B6 deficiency (<110 nmol/L), self-reported vitamin B supplement use up to 1 year prior to laboratory investigations, elevated (>3SD above the mean) blood levels of vitamin B12, folate or vitamin B6 suggestive of vitamin supplement use, or decreased renal function as indicated by age-adjusted estimated glomerular filtration rate **Setting**: NR **Number of participants**: 311 **Age (years), mean (SD)**: 63.0 (11.4) **Male sex, n (%)**: 235 (71.0)	**Index test**: Total vitamin B12 measured in plasma with Beckman Coulter DXI immunoenzymatic assay. **Reference tests**: (1) MMA; (2) Hcy; (3) Hcy and/or MMA. MMA was measured in plasma by chromatography-tandem mass spectroscopy. Hcy was measured with Atellica immunoassay.	**Outcomes**: Diagnostic test accuracy (threshold vitamin B12 levels to obtain 90% and 95% sensitivity, determined the optimum trade-off between sensitivity and specificity for the different reference metabolites, AUC) **Follow-up**: NA
Ao et al. (2017)[13] Japan **Funding**: JSPS KAKENHI	Cross-sectional	**Inclusion criteria**: Outpatients with Crohn's disease **Exclusion criteria**: NR **Setting**: Gastroenterology Clinic at Kyoto University Hospital **Number of participants**: 48 **Age (years), mean (SD)**: 40.1 (9.0) **Age (years), median**: 39.5 **Male sex, n (%)**: 33 (68.8)	**Index test**: Serum concentrations of vitamin B12 was measured by chemiluminescent enzyme immunoassay (CLEIA) **Reference test**: Plasma concentration of Hcy was measured by High-performance liquid chromatography (HPLC)	**Outcomes**: Diagnostic test accuracy (AUC) **Follow-up**: NA
Vashi et al. (2016)[14] USA **Funding**: No funding	Cross-sectional (consecutive case series)	**Inclusion criteria**: Cancer patients greater than 18 years of age **Exclusion criteria**: No exclusion criteria **Setting**: Cancer Treatment Centers of America (CTCA) at Midwestern Regional Medical Center **Number of participants**: 316 **Age (years), mean**: 52.5 **Age (years), median (range)**: 54 (22 to 82) **Male sex, n (%)**: 134 (42.4)	**Index test**: Vitamin B12 measured using the ARCHITECT B-12 assay, MMA measured using Liquid chromatography/ tandem mass spectrometry (LC/ MS-MS), Hcy measured using the Dimension Vista ® System **Reference test**: Fedosov quotient (combined vitamin B12, MMA, and Hcy)	**Outcomes**: Diagnostic test accuracy (AUC, Sensitivity, Specificity) **Follow-up**: NA

Study citation, country, funding source	Study design	Population characteristics	Intervention and comparator(s)	Clinical outcomes, length of follow-up
Beletić et al. (2015)[15] Serbia **Funding**: Ministry of Education, Science and Technological Development of the Republic of Serbia	Cross-sectional	**Inclusion criteria**: People with chronic obstructive pulmonary disease (COPD) diagnosed according to the Global Initiative for Chronic Obstructive Lung Disease **Exclusion criteria**: Smoking, alcohol consumption and supplementation with folic acid and/or vitamin B12, as well as the presence of coronary heart disease, cerebrovascular and gastrointestinal disorders, renal insufficiency, diabetes, malignant or autoimmune disease **Setting**: Clinic for Lung Diseases and the Center for Medical Biochemistry **Number of participants**: 50 **Age (years), mean (SD)**: 49.0 (14.5) **Male sex, n (%)**: 28 (56)	**Index test**: Hcy thresholds of 10, 12 and 15 µmol/L **Reference test**: Vitamin B12 thresholds of 203 ng/L and 473 ng/L The chemiluminescent micropartical immunoassays on the ARCHITECT® ci8200 Integrated System (Abbott Diagnostics, Wiesbaden, Germany) was applied for measurement of homocysteine and vitamin B12 concentrations.	**Outcomes**: Diagnostic test accuracy (AUC) **Follow-up**: NA
Cinemre et al. (2015)[16] Turkey **Funding**: Scientific Research Project Support Fund of Sakarya University	Cross-sectional	**Inclusion criteria**: Patients with myeloproliferative disorders **Exclusion criteria**: Those with gastrointestinal disorders such as Crohn disease, prior gastric or ileal resection, concurrent metformin use, a purely vegetarian diet, or serum creatinine levels greater than 1.1 mg/dL for women and greater than 1.3 mg/dL for men **Setting**: Hematology department **Number of participants**: 58 **Age (years), mean (SD)**: 61 (15) **Male sex, n (%)**: 29 (50)	**Index test:** (1) HoloTC measured using an automated commercial immunoassay (Architect i2000SR, Active B12; Abbott Laboratories, Abbott Park, IL) (2) Total plasma Hcy (Immulite 2000; Siemens Healthcare Di agnostics, Deerfield, IL) **Reference test**: Serum MMA measured using liquid chromatography-tandem mass spectrometry (HPLC 1200 binary pump and 1200 Autosampler; Agilent, Santa Clara, CA) and a detector (API 5500; ABSciex, Framingham, MA)	**Outcomes**: Diagnostic test accuracy (AUC) **Follow-up**: NA

AUC = area under the curve; Hcy = Homocystein; MMA = methylmalonic acid; NA = not applicable; NR = not reported; SD = standard deviation

Appendix 3: Critical Appraisal of Included Publications

Note that this appendix has not been copy-edited.

Table 4: Strengths and Limitations of Systematic Reviews Using AMSTAR 2[8]

Strengths	Limitations
Anastasi et al. (2021)[11]	
• The review methods were defined prior to undertaking the review, a protocol was written and reviewed by independent, anonymous peer reviewers • Eligibility criteria were clearly defined, and covered the elements of PICO and included study designs • Several electronic databases were searched, the search strategies were provided, and grey literature searching was performed • Study selection and risk of bias assessment were performed by two independent reviewers • A PRISMA flow diagram was provided • The overall strength of the evidence was evaluated using the GRADE approach	• Data extraction was performed by one reviewer, and verified by a second reviewer • A list of excluded studies and reason for exclusion was not provided

AMSTAR 2 = A MeaSurement Tool to Assess systematic Reviews 2; GRADE = Grading of recommendations, assessment, development, and evaluations; PICO = Participants, intervention, comparator, outcomes; PRIMSA = Preferred Reporting Items for Systematic Reviews and Meta-Analyses

Table 5: Strengths and Limitations of Diagnostic Test Accuracy Studies Using QUADAS-2

Strengths	Limitations
Warendorf et al. (2021)[12]	
• The objectives, index test, reference test, and main outcomes were clearly described • A case-control design was avoided • The index test and reference standard matched the review question • The inclusion and exclusion criteria were included • All participants received the same index and reference standard (within 60 days of each other) • The index and reference test were well described and the values for the cut-offs were pre-specified • 285 of 331 patients contributed to the DTA results, after excluding those with absolute vitamin B12 deficiency (<148 pmol/L) • The authors declared they had no conflict of interest regarding the publication of the article • Source of funding (i.e., no funding) was disclosed	• The methods of patient selection were not well described (i.e., consecutive or random sample). • It was unclear if the results of the index tests were read without knowledge of the results of the reference standard

Strengths	Limitations
Ao et al. (2017)[13]	
• The objectives, index test, reference test, and main outcomes were clearly described • A case-control design was avoided • The index test and reference standard matched the review question • The index and reference test were well described and the values for the cut-offs were pre-specified • All participants were included in the analysis • The authors declared they had no conflict of interest • Source of funding was disclosed	• The methods of patient selection were not well described (i.e., consecutive or random sample). • No description of the time interval between the index and reference test • It was unclear if the results of the index tests were read without knowledge of the results of the reference standard • Patients had Crohn's disease, which may not be generalizable to other populations suspected to be vitamin B12 deficient.
Vashi et al. (2016)[14]	
• The objectives, index test, reference test, and main outcomes were clearly described • A case-control design was avoided; a consecutive case series was used to select patients • The index test and reference standard matched the review question • The index and reference test were well described and the values for the cut-offs were pre-specified • Vitamin B12, MMA, Hcy were determined simultaneously in each patient • 223 of 316 patients contributed to the ROC analysis, after excluding patients with increased serum creatinine (1.2 mg/dl), patients who were malnourished, and patients with estimated glomerular filtration rate <60 ml/min/1.73m2 to reduce confounding • The authors declared they had no conflict of interest • Source of funding (i.e., no funding) was disclosed	• Each index test was also included in the reference test • Patients had cancer, which may not be generalizable to other populations suspected to be vitamin B12 deficient

Strengths	Limitations
Beletic et al. (2015)[15]	
• The objectives, index test, reference test, and main outcomes were clearly described • A case-control design was avoided • The index test and reference standard matched the review question • The inclusion and exclusion criteria were included • All participants received the same index and reference standard at the same time • All participants were included in the analysis • The index and reference test were well described and the values for the cut-offs were pre-specified • The same assay was used to determine the results for all participants, and for both Hcy and vitamin B12 • The authors declared they had no conflict of interest regarding the publication of the article • Source of funding was disclosed	• The methods of patient selection were not well described (i.e., consecutive or random sample) • It was unclear if the results of the index tests were read without knowledge of the results of the reference standard • Patients had chronic obstructive pulmonary disease, which may not be generalizable to other populations suspected to be vitamin B12 deficient
Cinemre et al. (2015)[16]	
• The objectives, index test, reference test, and main outcomes were clearly described • A case-control design was avoided • The index test and reference standard matched the review question • The inclusion and exclusion criteria were included • All participants received the same index and reference standard at the same time • All participants were included in the analysis • The index and reference test were well described and the values for the cut-offs were pre-specified • Source of funding was disclosed	• The methods of patient selection were not well described (i.e., consecutive or random sample) • It was unclear if the results of the index tests were read without knowledge of the results of the reference standard • Patients had myeloproliferative disorders, which may not be generalizable to other populations suspected to be vitamin B12 deficient • No statement around conflict of interest

Appendix 4: Main Study Findings and Authors' Conclusions

Note that this appendix has not been copy-edited.

Table 6: Summary of Findings Included Systematic Reviews

Main study findings	Authors' conclusion
Anastasi et al. (2021)[11]	

• No direct evidence evaluating the impact of B12 testing on patient-relevant outcomes was identified
• Setting, patient populations, diagnostic thresholds, index, and reference tests varied between studies
• Schwarz et al. (2015)
  ◦ Vitamin B12 (CLIA) (<156 pmol/L) versus holoTC (<50 pmol/L): Sens: 38%, Spec: 94%
  ◦ Vitamin B12 (MTP) (<212 pmol/L) versus holoTC (<50 pmol/L): Sens: 50%, Spec: 96%
  ◦ MMA (>0.30 µmol/L) versus holoTC (<50 pmol/L): Sens: 40%, Spec: 94%
  ◦ Vitamin B12 (CLIA) (<156 pmol/L) versus holoTC (<35 pmol/L): Sens: 53%, Spec: 93%
  ◦ Vitamin B12 (MTP) (<212 pmol/L) versus holoTC (<35 pmol/L): Sens: 71%, Spec: 95%
  ◦ MMA (>0.30 µmol/L) versus holoTC (<35 pmol/L): Sens: 41%, Spec: 90%
• Heil et al. (2012)
  ◦ Vitamin B12 (<146 pmol/L) versus MMA (>0.45 µmol/L): Sens: 53%, Spec: 81%
  ◦ Vitamin B12 (<146 pmol/L) versus holoTC (<21 pmol/L): Sens: 64%, Spec: 88%
• Schrempf et al. (2011)
  ◦ Vitamin B12 (<206 pmol/L) versus MMA (>0.298 µmol/L): Sens: 66.2%, Spec: 62.1%
  ◦ Vitamin B12 (<206 pmol/L) versus holoTC (<42 pmol/L): Sens: 56.3%, Spec: 50.5%
• Valente et al. (2011)
  ◦ Vitamin B12 (<123 pmol/L) versus Red cell cobalamin (<33 pmol/L): Sens: 33%, Spec: 95%
  ◦ holoTC (<20 pmol/L) versus Red cell cobalamin (<33 pmol/L): Sens: 55%, Spec: 96%
  ◦ MMA (>0.36 µmol/L) versus Red cell cobalamin (<33 pmol/L): Sens: 81%, Spec: 63%
  ◦ All diagnostic test accuracy studies were judged by the systematic review authors to be at risk of bias and the certainty of the evidence ranged from very low to low certainty
	• "The patient populations in the included studies were largely from samples of patients tested without a defined reason for testing. (p. 65)"[11]
	• There is no evidence directly measuring the impact of B12 tests on patient reported outcomes
	• The serum vitamin B12 test is prone to confounding factors
	• The current evidence does not provide enough information to determine the most appropriate test, or combination of tests, to use on patients with suspected or at high-risk of vitamin B12 deficiency
	• "Regardless of the first-line test, the total vitamin B12 or holoTC test often need to be supported with second-line testing in order to obtain highly sensitive and specific test results. (p. 63)"[11]

CLIA = chemiluminescent immunoassay; Hcy = Homocysteine; holoTC = Holotranscobalamin; MMA = Methymalonic acid; MTP = microbiological tests with microtitre plates

Table 7: Summary of Findings of Included Diagnostic Test Accuracy Studies

Main study findings	Authors' conclusion
Warendorft 2021[12]	
MMA > 0.29 µmol/L: AUC 0.72 • Best trade-off: 69% sensitivity, 66% specificity at a vitamin B12 threshold of 213 pmol/L • 97% sensitivity, 26% specificity at a vitamin B12 threshold of <304 pmol/L (412 pg/mL) • 91% sensitivity, 38% specificity at a vitamin B12 threshold level <264 pmol/L (358 pg/mL) Hcy > 14 µmol/L: AUC 0.61 • Best trade-off: 79% sensitivity, 44% specificity at a vitamin B12 threshold of 257 pmol/L • 96% sensitivity, 10% specificity at a vitamin B12 threshold of <374 pmol/L • 90% sensitivity, 21% specificity at a vitamin B12 threshold level <329 pmol/L MMA > 29 µmol/L and/or Hcy > 14 µmol/L: AUC 0.65 • Best trade-off: 80% sensitivity, 46% specificity at a vitamin B12 threshold of 257 pmol/L • 96% sensitivity, 10% specificity at a vitamin B12 threshold of <368 pmol/L • 90% sensitivity, 23% specificity at a vitamin B12 threshold level <327 pmol/L	• Deficiency is defined as vitamin B12 levels between 148 and 304 pmol/L with elevated MMA, and justify vitamin B12 supplementation • Measurements of Hcy levels added no value for supplementation • As MMA is a costlier assay, it should be restricted to those who are most likely to have an elevated MMA; which was a vitamin B12 level of <264 pmol/L (90% sensitivity) in this study • Vitamin B12 levels may differ between institutions, the methods of measurement, and the laboratories
Ao 2017[13]	
• AUC for vitamin B12 for hyperhomocysteinemia (HHcy) (>14 nmol/mL): 0.73 (95% CI: 0.606 to 0.900) • The adequate cut-off value for vitamin B12 was calculated to be 503 pg/mL based on Youden's index	• HHcy was prevalent in patients with Crohn's disease, probably due to vitamin B12 malabsorption • Serum vitamin B12, not folic acid, is the major determinant for HHcy, and a majority of the patients (68.8%) had serum vitamin B12 concentration below the cut-off value (503 pg/mL) based on prevention of HHcy

Main study findings	Authors' conclusion
Vashi 2016[14]	
• Using the cut-offs vitamin B12 (<300 pm/mL), MMA (>260 nmol/L), Hcy (>12 µmol/L), the AUC for predicting cB12 (i.e., combined vitamin B12, MMA, and Hcy) ◦ vitamin B12: 0.83 ◦ MMA: 0.98 ◦ Hcy: 0.85 • Vitamin B12 cut-off level of 385 pg/mL provided 86% sensitivity and 80% specificity to detect B12 deficiency • MMA cut-off level of 413.5 nmol/L provided 86% sensitivity and 99% specificity to detect B12 deficiency • Hcy cut-off level of 15.5 µmol/L provided 71% sensitivity and 95% specificity to detect B12 deficiency	• Vitamin B12 is poorly correlated with MMA and Hcy in cancer and can lead to significant under-diagnosis • There was a weak association between vitamin B12 with MMA and Hcy • "Given that individual tests lack enough sensitivity and specificity, we recommend that testing strategies for vitamin B-12 deficiency include B-12, MMA and HCy levels used in combination in patients suspected to have risk factors for vitamin B-12 deficiency as well as in patients in whom a definitive diagnosis of vitamin B-12 deficiency cannot be reached. (p. 10)"[14] • Patients who are identified through clinical assessment as having risk factors and/or symptoms suggestive of deficiency should have additional testing of MMA and Hcy regardless of their vitamin B12 levels
Beletic 2015[15]	
Results presented as AUC (SE) (95% CI); P value At a vitamin B12 cut-off level of 203 ng/L • Hcy 10 µmol/L: 0.553 (0.267) (0.029 to 1.000); 0.859 • Hcy 12 µmol/L: 0.632 (0.224) (0.193 to 1.000); 0.657 • Hcy 15 µmol/L: 0.842 (0.108) (0.631 to 1.000); 0.248 At a vitamin B12 cut-off level of 473 ng/L • Hcy 10 µmol/L: 0.633 (0.097) (0.443 to 0.824); 0.166 • Hcy 12 µmol/L: 0.671 (0.094) (0.487 to 0.854); 0.076 • Hcy 15 µmol/L: 0.504 (0.096) (0.316 to 0.693); 0.965	• ROC analyses did not show any statistically significance ($p<0.05$) of Hcy as a predictor for vitamin B12 deficiency, regardless of the cut-off used • B12 deficiency in COPD patients cannot be predicted by occurrence of HHcy

Main study findings	Authors' conclusion
Cinemre 2015[16]	
• Using the cut-offs holoTC (< 35 pmol/L), Hcy (>13 mol/L), MMA (20 to 39 years: > 0.27 nmol/mL; 40 to 59 years: > 0.30 nmol/mL; 60+ years: > 0.45 nmol/mL), the AUC (SE) *Z* (95%CI); p-value were: ◦ HoloTC: 0.822 (0.057) 5.64 (0.70 to 0.91); p≤ 0.001 ◦ Hcy: 0.662 (0.0.74) 2.18 (0.53 to 0.78); p=0.03 • HoloTC cut-off ≤ 40.6 pmol/L: ◦ Sensitivity (95% CI): 75 (59 to 87) ◦ Specificity (95% CI): 78 (52 to 94) ◦ Likelihood Ratio Positive (95% CI): 3.4 (1.4 to 8) ◦ Likelihood Ratio Negative (95% CI): 0.3 (0.2 to 0.6) ◦ Positive Predictive Value (95% CI): 88 (73 to 97) ◦ Negative Predictive Value (95% CI): 58 (37 to 78) • Hcy cut-off > 14 mol/L: ◦ Sensitivity (95% CI): 70 (54 to 81) ◦ Specificity (95% CI): 61 (36 to 83) ◦ Likelihood Ratio Positive (95% CI): 1.8 (1 to 3.3) ◦ Likelihood Ratio Negative (95% CI): 0.5 (0.3 to 0.9) ◦ Positive Predictive Value (95% CI): 80 (63 to 92) ◦ Negative Predictive Value (95% CI): 48 (27 to 69)	• AUC values for holoTC and Hcy were both statistically significant (p<0.05) when compared to the age-adjusted MMA levels • HoloTC had greater sensitivity, specificity, positive likelihood ratio, positive predictive value, and negative predictive value than for Hcy • "The holoTC level may replace conventional vitamin B12 level as the optimal initial test for this patient group and may replace vitamin B12 level as a second direct test for metabolic indicators. (p. 640)"[16]

AUC = Area Under the Curve; CI = Confidence interval; Hcy = Homocysteine; HHcy = Hyperhomocysteinemia; HoloTC = Holotranscobalamin; MMA = Methylmalonic Acid; SE = Standard Error

Appendix 5: References of Potential Interest

Note that this appendix has not been copy-edited.

Previous CADTH Reports

Vitamin B12 testing in the general population: clinical and cost-effectiveness. CADTH rapid response report: summary of abstracts Ottawa (ON): CADTH; 2015: https://www.cadth.ca/sites/default/files/pdf/htis/jan-2015/RB0781%20Vitamin%20B12%20Testing%20Final.pdf. Accessed 2022 Feb 25

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