National Institute for Health and Care Excellence

Draft for consultation

Vitamin B12 deficiency in over 16s: diagnosis and management

[C] Evidence review for diagnostic tests

NICE guideline <number>

Evidence reviews underpinning recommendations 1.3.1 to 1.3.15 and research recommendations in the NICE guideline

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Draft for Consultation

Developed by NICE

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Diagnosing vitamin B12 deficiency

1.1 Review question

What is the diagnostic accuracy of tests (including the serum cobalamin assay and holotranscobalamin, methylmalonic acid and homocysteine tests) for diagnosing vitamin B12 deficiency?

1.1.1 Introduction

An accurate test of vitamin B12 status is important in supporting a diagnosis of deficiency. The prompt diagnosis and correction of vitamin B12 deficiency guards against the development of megaloblastic anaemia, potentially irreversible neuropathy and neuropsychiatric changes. Definitive exclusion of vitamin B12 deficiency is also important because it allows for alternative causes of symptoms to be investigated.

Blood tests help establish a diagnosis of deficiency in the presence of recognised signs and symptoms, but there is no gold standard clinical or biochemical test available. The concentration of cobalamin (total B12 test), holotranscobalamin (also known as 'active B12'), methylmalonic acid (MMA) and total homocysteine in the blood can all be used to diagnose vitamin B12 deficiency, although the accuracy of each biomarker may vary between different patient groups. There is no single, widely adopted diagnostic algorithm for the diagnosis of vitamin B12 deficiency. Different approaches are taken both for initial testing and in the application of subsequent confirmatory tests where results fall into a range of diagnostic uncertainty.

This review aims to determine which way of diagnosing vitamin B12 deficiency is most accurate and leads to the best outcomes for patients.

1.1.2 Summary of the protocol

For full details see the review protocol in Appendix A.

Table 1: PICO characteristics of review question

Population	Inclusion: Adults with suspected vitamin B12 deficiency Exclusion: people taking vitamin B12 supplements Strata: • Age (adults 16/18 years and older; older adults 65 years and older) • Third trimester of pregnancy (third trimester; first two trimesters and not pregnant) • Ethnicity (Afro-Caribbean; other) • Sex (male; female) (study defined) for homocysteine test only
Target condition	Vitamin B12 deficiency
Index tests	The following as stand-alone tests, in combination or as staged tests: • Serum cobalamin assay • Holotranscobalamin test • Methylmalonic acid test (including urinary) • Homocysteine test

	Strata: reference ranges as defined by the studies
Reference standards	Reference standards defined by the studies
Statistical measures	• Sensitivity 90% for first line and 80% for second line tests • Specificity 70% for first line and 90% for second line tests • Raw data to calculate 2x2 tables to calculate sensitivity and specificity (number of true positives, true negatives, false positives, and false negatives). • Predictive values • Likelihood ratios
Study design	Inclusion: • Cross-sectional studies • Diagnostic accuracy observational cohort studies • Systematic reviews of the above Exclusion: Case-control studies

1.1.3 Methods and process

This evidence review was developed using the methods and process described in Developing NICE guidelines: the manual. Methods specific to this review question are described in the review protocol in appendix A and the methods document.

Declarations of interest were recorded according to NICE's conflicts of interest policy.

1.1.4 Diagnostic evidence

1.1.4.1 Included studies

Ten diagnostic accuracy observational cohort studies (12 papers) were included in the review;[1-8, 10, 11, 13, 19] these are summarised in Table 2 below. Evidence from these studies is summarised in the clinical evidence summary tables below (Table 3, Table 4, Table 5, Table 6, Table 7, Table 8, Table 9, Table 10, Table 11). The assessment of the evidence quality was conducted with emphasis on test sensitivity for first line tests and specificity for second line tests as these were identified by the committee as the primary measures in guiding decision-making. The committee set clinical decision thresholds for first line tests as sensitivity/specificity 90% and 70% above which a test would be recommended and 60% and 40% below which a test is of no clinical use. The committee set clinical decision thresholds for second line tests as sensitivity/specificity 80% and 90% above which a test would be recommended and 50% and 60% below which a test is of no clinical use.

See also the study selection flow chart in Appendix C, sensitivity and specificity forest plots in Appendix E, and study evidence tables in Appendix D.

A search was conducted for cross-sectional and prospective and retrospective cohort studies assessing the diagnostic test accuracy of the serum cobalamin assay, holotranscobalamin test, methylmalonic acid test and homocysteine test to identify whether vitamin B12 deficiency is present (as indicated by the reference standard) in people under investigation for vitamin B12 deficiency. Ten studies (12 papers) were identified.

All studies were in mixed (adults ≥16/18 years and older adults ≥65 years) or unclear adult populations. No evidence was identified for women and people in the third trimester of pregnancy, Afro-Caribbean ethnicity or for separate genders (for homocysteine test).

Evidence was identified for all index tests. Seven studies reported the diagnostic accuracy of the serum cobalamin assay, six studies reported the diagnostic accuracy of holotranscobalamin, four studies reported the diagnostic accuracy of methylmalonic acid and four studies reported the diagnostic accuracy of homocysteine. A variety of cut-offs were used for all index tests (see Table 2). No two studies used the same reference standard, i.e., the same test at the same threshold for defining test positivity, and some studies used more than one reference standard to identify probable or borderline deficiency.

The majority of the evidence identified was for the use of the index tests as first line tests. Two studies reported the diagnostic accuracy of index tests as second line tests after a total B12 test showed low total B12 concentration.

1.1.4.2 Excluded studies

The committee were aware of a published Medtech innovation briefing[14] developed by NICE on the Active-B12 assay for diagnosing vitamin B12 deficiency. The diagnostic accuracy was evaluated against various reference standards reported in the studies. All included studies were cross-checked for inclusion in this review as relevant.

See the excluded studies list in Appendix J.

1.1.5 Summary of studies included in the diagnostic evidence

Table 2: Summary of studies included in the evidence review

Study	Population	Index test	Reference standard	Comments
Bolann 2000 [1]	N=196 subjects referred to the Department of Clinical Chemistry at a single hospital by general practitioners for determination of serum cobalamin between June 1994 and November 1996 Age, median (range): 59 (17-87) years Pregnancy third trimester: not reported Ethnicity: not reported Gender: mixed. 67.9% female Vitamin B12 supplements: not reported	First line: Serum cobalamin (cut off 116 pmol/L) Serum cobalamin (cut off 150 pmol/L) Plasma total homocysteine (cut off 15 µmol/L) Plasma total homocysteine (cut off 11.3 µmol/L)	Initial MMA values >0.26 µmol/L (upper reference limit), which subsequently decreased by at least 50% 4 weeks after the start of cobalamin injections (1mg of cyanocobalamin intramuscularly twice weekly for 2.5 weeks)	Study conducted in Norway TP, FP, TN, FN values and CIs calculated from reported sensitivity/specificity and prevalence data
Bondu 2020 [2]	N=217 patients attending the outpatient department at a Medical College, with a total Vitamin B12 lab request Age, mean (standard deviation), range: 44.5 (13.7), 17-83 years Pregnancy third trimester: not reported	First line: Holotranscobalamin, cut off not reported Total Homocysteine, cut off not reported	Deficient: serum total vitamin B12 levels below 200 pg/mL Borderline: serum total vitamin B12 levels ranging from 200 to 350 pg/mL	Study conducted in India Cut off values for index tests not reported

Study	Population	Index test	Reference standard	Comments
	Ethnicity: not reported Gender: mixed. 54.4% female Vitamin B12 supplements: not reported		Sufficient: serum total vitamin B12 levels >350 pg/mL	Results reported for deficiency and deficiency/borderline deficiency Sensitivity/specificity calculated from 2x2 tables
Campos 2020 [8]; Campos 2020 [3]	Study 1: n = 11,833 samples from 9,464 patients Consecutive routine measurement results from investigations of vitamin B12 status in 2 medical laboratories Those with simultaneous measurement of B12, HoloTC, MMA and Hcy were included in the analysis Age: median (IQR) suggest majority were adults (56 (41-68) years) Pregnancy third trimester: not reported Ethnicity: not reported Gender: mixed. 58.8% female Vitamin B12 supplements: not reported	First line: HoloTC (cut off <27 pmol/L for possible/probable deficiency, <45 pmol/L for subclinical deficiency) HoloTC (cut off <56.5 pmol/L for possible/probable deficiency, <73 pmol/L for subclinical deficiency) HoloTC (cut off <19 pmol/L for possible/probable deficiency, <25 pmol/L for subclinical deficiency) B12 (cut off <167 pmol/L for possible/probable deficiency, <229 pmol/L for subclinical deficiency) B12 (cut off <320 pmol/L for possible/probable deficiency, <351 pmol/L for subclinical deficiency)	4cB12 Integrates the direct markers (HoloTC and B12) in pmol/L and metabolic markers (MMA and Hcy) in µmol/L of B12 deficiency and age based on models obtained from large empirical investigations 4cB12 ≤−0:5 was defined as an indicator of low B12, with at least potential subclinical manifestations of B12 deficiency. A value <−1:5 indicates possible and probable B12 deficiency	Retrospective cohort study conducted in Switzerland and Liechtenstein Results reported for possible/probable deficiency (4cB12 ≤ -1.5) and subclinical deficiency (4cB12 ≤ -0.5 and >-1.5) Separate analyses reported for males and females and age < 50 and age ≥ 50 years, but AUCs only

Study	Population	Index test	Reference standard	Comments
		B12 (cut off <115 pmol/L for possible/probable deficiency, <142 pmol/L for subclinical deficiency) MMA (cut off >466 nmol/L for possible/probable deficiency, >245 nmol/L for subclinical deficiency) MMA (cut off >158 nmol/L for possible/probable deficiency, >152 nmol/L for subclinical deficiency) MMA (cut off >723 nmol/L for possible/probable deficiency, >480 nmol/L for subclinical deficiency) Homocysteine (cut off >16.4 µmol/L for possible/probable deficiency, >15 µmol/L for subclinical deficiency) Homocysteine (cut off >6.2 µmol/L for possible/probable deficiency, >8 µmol/L for subclinical deficiency) Homocysteine (cut off >34 µmol/L for possible/probable deficiency, >29 µmol/L for		

Study	Population	Index test	Reference standard	Comments
		subclinical deficiency)		
	Study 2: n=3,614 samples from 3,333 patients Consecutive routine measurement results from investigations of vitamin B12 status in 2 medical laboratories Those with simultaneous measurement of B12, HoloTC, MMA, Hcy and folate were included in the analysis Age: median (IQR) suggest majority were adults (53 (40-64) years) Pregnancy third trimester: not reported Ethnicity: not reported Gender: mixed. 54.9% female Vitamin B12 supplements: not reported	First line (combination): $2cB12_{HoloTC/MMA}$ $2cB12_{B12/MMA}$ $2cB12_{B12/Hcy}$ $2cB12_{HoloTC/B12}$ $2cB12_{HoloTC/Hcy}$ $2cB12_{MMA/Hcy}$ $3cB12_{HoloTC/B12/MMA}$ $3cB12_{MMA/HoloTC/Hcy}$ $3cB12_{HoloTC/B12/Hcy}$ $3cB12_{MMA/B12/Hcy}$ First and second line (2 step algorithm): Harrington's algorithm – 2-step diagnostic algorithm. If HoloTC is 25-70 pmol/L, a subsequent measurement of MMA is performed. If MMA is <280 nmol/L (or <360 nmol/L in patients aged >65 years), vitamin B12 sufficiency is assumed, whereas MMA ≥280 nmol/L (≥360 nmol/L in patients ≥65 years), vitamin		

Study	Population	Index test	Reference standard	Comments
		B12 deficiency is postulated		
Goringe 2006 [4]	N=49 patients recruited from those referred to the Haematology laboratory of an NHS trust for B12 estimation and serum B12 <170 ng/L (n=27 with low Hb concentration and/or macrocytosis included in the analysis) Age: mixed. <75 years (no further information reported) Pregnancy third trimester: not reported Ethnicity: not reported Gender: mixed. 71.4% female Vitamin B12 supplements: not reported	Second line: Methylmalonic acid (cut off >0.47 µmol/L) Holotranscobalamin (cut off <38 pmol/L) Homocysteine (cut off >15 µmol/L)	Response to treatment with intramuscular B12 injections (1mg per week for 4 weeks), defined as an increase in Hb concentration of 1 g/dL or more and/or a decrease in MCV of 3 fl or more. Patients reassessed at 3 months.	Study conducted in Wales Those with abnormal liver function tests, hypothyroidism, alcohol abuse, folate deficiency, or renal failure were excluded Sensitivity/specificity calculated from 2x2 tables Authors report that symptomatic improvement did not correlate with hematologic response to treatment
Heil 2012 [5]	n=360 patient samples collected from clinical chemistry laboratories at 5 hospitals. Each centre aimed to collect samples of 250 patients of whom vitamin B12 was requested - 20 with vitamin B12 concentration <100 pmol/L, 80 with vitamin B12 100 - 200 pmol/L, 100 with vitamin B12 200 - 300 pmol/L and 50 with vitamin B12 > 300 pmol/L Age, mean (range): mixed. 59 (19-100) Pregnancy third trimester: not reported	First line: Serum vitamin B12 (cut off <145 pmol/L) Serum vitamin B12 (cut off <180 pmol/L HoloTC (cut off <21 pmol/L) HoloTC (cut off <32 pmol/L)	Serum MMA >0.45 µmol/L (reference range 0.09 –0.45 µmol/L).	Study conducted in the Netherlands Only those with normal renal function were included

Study	Population	Index test	Reference standard	Comments
	Ethnicity: not known Gender: mixed. 62.2% female Vitamin B12 supplements: not reported			
Herrmann 2013 [6]	n = 1359 samples referred to a single laboratory for total vitamin B12 measurement. Samples were anonymous and no clinical information available Age, median (10-90th percentiles): mixed. Reported by percentile of holoTC, ranging from 51 (25-76) years to 71 (47-87) years Pregnancy third trimester: not reported Ethnicity: not reported Gender: not reported Vitamin B12 supplements: not reported	First line: Serum total vitamin B12 (cut off 227 pM) Holotranscobalamin (cut off 35 pM) Holotranscobalamin (cut off 22 pM) (in samples with serum creatinine ≤ 97.2 µM) Holotranscobalamin (cut off 76 pM) (in samples with serum creatinine ≤ 97.2 µM)	Methylmalonic acid >300 nM	Study conducted in Germany
Hollelan d 1999 [7]	n = 376 (n=224 included in the analysis) patients with s-cobalamin concentrations <300 pmol/L from a total of 76,840 cobalamin analyses performed at a single laboratory. Approximately 75 patients in each of the following serum cobalamin concentrations were included: 0–139, 140–169, 170–189, 190–219, and 220–299 pmol/L. Only one patient per general practitioner was included Age, median (range): mixed. Medians reported by serum cobalamin interval ranging from 59 to 69 (18-90) years Pregnancy third trimester: not reported Ethnicity: not reported Gender: mixed Female/male ratio	First line: Serum cobalamin (cut off ≤170 pmol/L)	Methylmalonic acid >0.376 µmol/L	Retrospective cohort study conducted in Norway Serum cobalamin ordered as a confirmation test of either an earlier s-cobalamin determination or as therapy control in patients on cobalamin supplementation were excluded

Study	Population	Index test	Reference standard	Comments
	reported by serum cobalamin interval 1.3, 1.6, 2.9, 1.5, 1.9 Vitamin B12 supplements: not reported but s-cobalamin ordered as therapy control in patients on cobalamin supplementation was excluded			
Matchar 1987 [10]; Matchar 1994 [11]	N=136 (n=96 with evaluable MMA results, complete follow up and clinical diagnosis included in the MMA analysis; n=134 with complete follow up and clinical diagnosis included in the vitamin B12 analysis) patients having serum B12 levels measured. Age, mean (standard deviation): 61.6 (11.7) years Pregnancy third trimester: not reported Ethnicity: 69% white Gender: 2% female Setting: single veterans administration medical centre Country: USA Inclusion criteria: patients with low serum B12 levels (<180 pg/mL) and a random sample of patients with normal serum B12 assay results matched by assay date Exclusion criteria: living >1 hour from the hospital and could not reliably keep follow up appointments, died before first evaluation Vitamin B12 supplements: not reported	First line: Urinary MMA (cut off 5µg/mg creatinine) Serum cobalamin (cut off <133 pmol/L)	All abnormalities suggestive of deficiency or fewer abnormalities if lessened in response to treatment with B12	Prospective cohort study conducted in USA
Moelby 1990 [13]	n=42 patients undergoing haematological evaluation for cobalamin deficiency with serum cobalamin levels <100 pmol l-1. Age, range: 24-84 years Pregnancy third trimester: not reported Ethnicity: not reported Gender: 83% female	Second line: Serum MMA (cut off >0.34 µmol l-1)	Serum cobalamin <100 pmol l-1 and abnormal Schilling test and/or megaloblastic bone marrow morphology which couldn't be explained by folate deficiency	Prospective cohort study conducted in Denmark

Study	Population	Index test	Reference standard	Comments
	Vitamin B12 supplements: not reported			
Schrempf 2011 [19]	n = 1,279 subjects admitted to the Department of Neurology with neuropsychiatric conditions suspicious for VitB12 deficiency (only those with normal renal function were included in the main analysis, n=851) Age, mean (standard deviation): mixed 67.7 (15.2), range: 18–98 years (65.7 ± 15.2 [18–98] years in those with normal renal function) Pregnancy third trimester: not reported Ethnicity: not reported Gender: 48.9% female (72.7% in the those with normal renal function) Vitamin B12 supplements: data regarding VitB12 supplementation or intake not available	First line: Serum vitamin B12 (cut off <211 pg/ml) Serum vitamin B12 (cut off <280 pg/ml) Serum vitamin B12 (cut off <395 pg/ml) HoloTC (cut off <19 pmol/l) HoloTC (cut off <42 pmol/l) HoloTC (cut off <67 pmol/l)	MMA > 47 µg/l	Retrospective cohort study conducted in Germany Main analysis restricted to the cohort with normal renal function. Data on the overall patient cohort including those with abnormal renal function is presented in supplementary material

1 See Appendix D for full evidence tables.

2 1.1.6 Summary of the diagnostic evidence

3 The assessment of the evidence quality was conducted with emphasis on test sensitivity for
4 first line tests and specificity for second line tests as these were identified by the committee
5 as the primary measures in guiding decision-making. The committee set clinical decision
6 thresholds for first line tests as sensitivity/specificity 90% and 70% above which a test would
7 be recommended and 60% and 40% below which a test is of no clinical use. The committee
8 set clinical decision thresholds for second line tests as sensitivity/specificity 80% and 90%
9 above which a test would be recommended and 50% and 60% below which a test is of no
10 clinical use.

11 **Table 3: Clinical evidence summary: serum cobalamin assay (first line)**

Studies	N	Risk of bias	Inconsistency	Indirectness	Imprecision	Effect size (95%CI)	Quality
Serum cobalamin <116 pmol/L for diagnosing deficiency (MMA response to treatment)							
1 diagnostic accuracy observational cohort study	187	Serious[1]	Not serious	Very serious[2]	Serious[3]	Sensitivity=0.73 (0.58-0.84)	VERY LOW
		Serious[1]	Not serious	Very serious[2]	Serious[4]	Specificity=0.74 (0.66-0.81)	VERY LOW
Serum cobalamin <150 pmol/L for diagnosing deficiency (MMA response to treatment)							

Studies	N	Risk of bias	Inconsistency	Indirectness	Imprecision	Effect size (95%CI)	Quality
1 diagnostic accuracy observational cohort study	187	Serious[1]	Not serious	Very serious[2]	Serious[5]	Sensitivity=0.90 (0.79-0.97)	VERY LOW
		Serious[1]	Not serious	Very serious[2]	Not serious	Specificity=0.60 (0.52-0.69)	VERY LOW
B12 <167 pmol/L for diagnosing possible/probable deficiency (4cB12 ≤ -1.5)							
1 diagnostic accuracy retrospective observational cohort study	11833	Serious[6]	Not serious	Very serious[2]	Serious[5]	Sensitivity=0.95 (0.86-0.99)	VERY LOW
		Serious[6]	Not serious	Very serious[2]	Not serious	Specificity=0.92 (0.92-0.93)	VERY LOW
B12 <320 pmol/L for diagnosing possible/probable deficiency (4cB12 ≤ -1.5)							
1 diagnostic accuracy retrospective observational cohort study	11833	Serious[6]	Not serious	Very serious[2]	Not serious	Sensitivity=0.98 (0.91-1.00)	VERY LOW
		Serious[6]	Not serious	Very serious[2]	Not serious	Specificity=0.42 (0.41-0.42)	VERY LOW
B12 <115 pmol/L for diagnosing possible/probable deficiency (4cB12 ≤ -1.5)							
1 diagnostic accuracy retrospective observational cohort study	11833	Serious[6]	Not serious	Very serious[2]	Serious[3]	Sensitivity=0.57 (0.43-0.70)	VERY LOW
		Serious[6]	Not serious	Very serious[2]	Not serious	Specificity=0.99 (0.99-0.99)	VERY LOW
B12 <229 pmol/L for diagnosing subclinical deficiency (4cB12 ≤ -0.5 and >-1.5)							
1 diagnostic accuracy retrospective observational cohort study	11833	Serious[6]	Not serious	Very serious[2]	Not serious	Sensitivity=0.86 (0.84-0.88)	VERY LOW
		Serious[6]	Not serious	Very serious[2]	Not serious	Specificity=0.78 (0.77-0.78)	VERY LOW
B12 <351 pmol/L for diagnosing subclinical deficiency (4cB12 ≤ -0.5 and >-1.5)							
1 diagnosti		Serious[6]	Not serious	Very serious[2]	Not serious	Sensitivity=0.99 (0.98-1.00)	VERY LOW

Studies	N	Risk of bias	Inconsistency	Indirectness	Imprecision	Effect size (95%CI)	Quality
c accuracy retrospective observational cohort study	11833	Serious[6]	Not serious	Very serious[2]	Not serious	Specificity=0.37 (0.36-0.38)	VERY LOW
B12 <142 pmol/L for diagnosing subclinical deficiency (4cB12 ≤ -0.5 and >-1.5)							
1 diagnostic accuracy retrospective observational cohort study	11833	Serious[6]	Not serious	Very serious[2]	Not serious	Sensitivity=0.28 (0.25-0.31)	VERY LOW
		Serious[6]	Not serious	Very serious[2]	Not serious	Specificity=0.99 (0.99-0.99)	VERY LOW
Serum vitamin B12 <145 pmol/L for diagnosing metabolic deficiency (MMA >0.45 µmol/L)							
1 diagnostic accuracy observational cohort study	360	Serious[7]	Not serious	Very serious[2]	Serious[3]	Sensitivity=0.53 (0.38-0.68)	VERY LOW
		Serious[7]	Not serious	Very serious[2]	Not serious	Specificity=0.81 (0.76-0.85)	VERY LOW
Serum vitamin B12 <180 pmol/L for diagnosing metabolic deficiency (MMA >0.45 µmol/L)							
1 diagnostic accuracy observational cohort study	360	Serious[7]	Not serious	Very serious[2]	Serious[3]	Sensitivity=0.64 (0.49-0.77)	VERY LOW
		Serious[7]	Not serious	Very serious[2]	Not serious	Specificity=0.64 (0.58-0.69)	VERY LOW
Serum vitamin B12 <227 pM for diagnosing deficiency (MMA >300 nM)							
1 diagnostic accuracy observational cohort study	1359	Serious[8]	Not serious	Very serious[2]	Not serious	Sensitivity=0.72 (0.67-0.76)	VERY LOW
		Serious[8]	Not serious	Very serious[2]	Serious[9]	Specificity=0.41 (0.38-0.44)	VERY LOW
Serum cobalamin ≤170 pmol/L for diagnosing functional deficiency (MMA >0.376 µmol/L)							
1 diagnostic accuracy retrospective observational	224	Serious[10]	Not serious	Very serious[2]	Serious[3]	Sensitivity=0.43 (0.10-0.82)	VERY LOW
		Serious[10]	Not serious	Very serious[2]	Not serious	Specificity=0.98 (0.95-0.99)	VERY LOW

Studies	N	Risk of bias	Inconsistency	Indirectness	Imprecision	Effect size (95%CI)	Quality
cohort study							
Serum vitamin B12 <180 pg/mL for diagnosing clinical deficiency (all abnormalities suggesting deficiency, or fewer if lessened in response to treatment)							
1 diagnostic accuracy prospective observational cohort study	134	Serious[11]	Not serious	Very serious[2]	Serious[5]	Sensitivity=1.00 (0.79-1.00)	VERY LOW
		Serious[11]	Not serious	Very serious[2]	Not serious	Specificity=0.53 (0.43-0.62)	VERY LOW
Serum vitamin B12 <211 pg/ml for diagnosing metabolic deficiency (MMA >47 µg/l) (normal renal function)							
1 diagnostic accuracy retrospective observational cohort study	851	Very serious[12]	Not serious	Very serious[2]	Not serious	Sensitivity=0.38 (0.27-0.50)	VERY LOW
		Very serious[12]	Not serious	Very serious[2]	Not serious	Specificity=0.87 (0.83-0.90)	VERY LOW
Serum vitamin B12 <280 pg/ml for diagnosing metabolic deficiency (MMA >47 µg/l) (normal renal function)							
1 diagnostic accuracy retrospective observational cohort study	851	Very serious[12]	Not serious	Very serious[2]	Serious[3]	Sensitivity=0.66 (0.54-0.77)	VERY LOW
		Very serious[12]	Not serious	Very serious[2]	Not serious	Specificity=0.62 (0.58-0.67)	VERY LOW
Serum vitamin B12 <395 pg/ml for diagnosing metabolic deficiency (MMA >47 µg/l) (normal renal function)							
1 diagnostic accuracy retrospective observational cohort study	851	Very serious[1]	Not serious	Very serious[2]	Serious[5]	Sensitivity=0.90 (0.81-0.96)	VERY LOW
		Very serious[1]	Not serious	Very serious[2]	Not serious	Specificity=0.35 (0.31-0.40)	VERY LOW
Serum vitamin B12 <211 pg/ml for diagnosing metabolic deficiency (MMA >47 µg/l) (whole cohort)							
1 diagnostic	1279	Very serious[12]	Not serious	Very serious[2]	Not serious	Sensitivity=0.34 (0.25-0.44)	VERY LOW

Studies	N	Risk of bias	Inconsistency	Indirectness	Imprecision	Effect size (95%CI)	Quality
accuracy retrospective observational cohort study		Very serious[1] [2]	Not serious	Very serious[2]	Not serious	Specificity=0.88 (0.85-0.90)	VERY LOW
Serum vitamin B12 <280 pg/ml for diagnosing metabolic deficiency (MMA >47 µg/l) (whole cohort)							
1 diagnostic accuracy retrospective observational cohort study	1279	Very serious[1] [2]	Not serious	Very serious[2]	Serious[3]	Sensitivity=0.63 (0.53-0.72)	VERY LOW
		Very serious[1] [2]	Not serious	Very serious[2]	Not serious	Specificity=0.64 (0.61-0.68)	VERY LOW
Serum vitamin B12 <630 pg/ml for diagnosing metabolic deficiency (MMA >47 µg/l) (whole cohort)							
1 diagnostic accuracy retrospective observational cohort study	1279	Very serious[1] [2]	Not serious	Very serious[2]	Serious[5]	Sensitivity=0.95 (0.89-0.98)	VERY LOW
		Very serious[1] [2]	Not serious	Very serious[2]	Not serious	Specificity=0.09 (0.07-0.12)	VERY LOW

[1] Serious risk of bias due to lack of reporting as to whether index tests and reference standard were conducted and interpreted without knowledge of each other and the time interval between their measurement.

[2] Evidence was downgraded by 1 increment if the majority of studies included an indirect population, and by 2 increments if the majority of studies included a very indirect population.

[3] Confidence interval crossed the decision threshold corresponding to 'low sensitivity' (60%).

[4] Confidence interval crossed the decision threshold corresponding to 'high specificity' (70%).

[5] Confidence interval crossed the decision threshold corresponding to 'high sensitivity' (90%).

[6] Serious risk of bias due to patient selection bias (analysis based on samples rather than patients).

[7] Serious risk of bias due to unclear method of patient selection and lack of reporting as to whether index tests and reference standard were conducted and interpreted without knowledge of each other.

[8] Serious risk of bias due to lack of reporting on methods of patient selection, patient characteristics and whether index tests and reference standard were conducted and interpreted without knowledge of each other.

[9] Confidence interval crossed the decision threshold for 'low specificity' (40%).

[10] Serious risk of bias due to methods of patient selection and lack of reporting regarding whether index tests and reference standard were conducted and interpreted without knowledge of each other.

[11] Serious risk of bias due to time interval between index test and reference standard.

[12] Very serious risk of bias due to lack of reporting regarding whether index tests and reference standard were conducted and interpreted without knowledge of each other and the time interval between index tests and reference standard and high number of participants excluded from the analysis with little explanation.

Table 4: Clinical evidence summary: holotranscobalamin (first line)

Studies	N	Risk of bias	Inconsistency	Indirectness	Imprecision	Effect size (95%CI)	Quality
Holotranscobalamin for diagnosing B12 deficiency (serum B12 <200 pg/mL)							
1 diagnostic accuracy observational cohort study	217	Very serious[1]	Not serious	Very serious[2]	Serious[3]	Sensitivity=0.84 (0.74-0.92)	VERY LOW
		Very serious[1]	Not serious	Very serious[2]	Serious[4]	Specificity=0.76 (0.68-0.83)	VERY LOW
Holotranscobalamin for diagnosing B12 deficiency and borderline deficiency (serum B12 ≤350 pg/mL							
1 diagnostic accuracy observational cohort study	217	Very serious[1]	Not serious	Very serious[2]	Serious[5]	Sensitivity=0.55 (0.47-0.62)	VERY LOW
		Very serious[1]	Not serious	Very serious[2]	Not serious	Specificity=0.98 (0.89-1.00)	VERY LOW
Holotranscobalamin <27 pmol/L for diagnosing possible/probable deficiency (4cB12 ≤ -1.5)							
1 diagnostic accuracy retrospective observational cohort study	1183	Serious[6]	Not serious	Very serious[2]	Serious[3]	Sensitivity=0.93 (0.83-0.98)	VERY LOW
		Serious[6]	Not serious	Very serious[2]	Not serious	Specificity=0.96 (0.96-0.96)	VERY LOW
Holotranscobalamin <56.5 pmol/L for diagnosing possible/probable deficiency (4cB12 ≤ -1.5)							
1 diagnostic accuracy retrospective observational cohort study	1183	Serious[6]	Not serious	Very serious[2]	Not serious	Sensitivity=0.98 (0.91-1.00)	VERY LOW
		Serious[6]	Not serious	Very serious[2]	Not serious	Specificity=0.60 (0.59-0.61)	VERY LOW
Holotranscobalamin <19 pmol/L for diagnosing possible/probable deficiency (4cB12 ≤ -1.5)							
1 diagnostic accuracy retrospective observational cohort study	1183	Serious[6]	Not serious	Very serious[2]	Not serious	Sensitivity=0.78 (0.65-0.87)	VERY LOW
		Serious[6]	Not serious	Very serious[2]	Not serious	Specificity=0.99 (0.99-0.99)	VERY LOW
Holotranscobalamin <45 pmol/L for diagnosing subclinical deficiency (4cB12 ≤ -0.5 and >-1.5)							

Studies	N	Risk of bias	Inconsistency	Indirectness	Imprecision	Effect size (95%CI)	Quality
1 diagnostic accuracy retrospective observational cohort study	1183 3	Serious[6]	Not serious	Very serious[2]	Not serious	Sensitivity=0.86 (0.83-0.88)	VERY LOW
		Serious[6]	Not serious	Very serious[2]	Not serious	Specificity=0.81 (0.80-0.82)	VERY LOW
Holotranscobalamin <73 pmol/L for diagnosing subclinical deficiency (4cB12 ≤ -0.5 and >-1.5)							
1 diagnostic accuracy retrospective observational cohort study	1183 3	Serious[6]	Not serious	Very serious[2]	Not serious	Sensitivity=0.99 (0.98-1.00)	VERY LOW
		Serious[6]	Not serious	Very serious[2]	Not serious	Specificity=0.44 (0.43-0.45)	VERY LOW
Holotranscobalamin <25 pmol/L for diagnosing subclinical deficiency (4cB12 ≤ -0.5 and >-1.5)							
1 diagnostic accuracy retrospective observational cohort study	1183 3	Serious[6]	Not serious	Very serious[2]	Not serious	Sensitivity=0.28 (0.25-0.30)	VERY LOW
		Serious[6]	Not serious	Very serious[2]	Not serious	Specificity=0.99 (0.99-0.99)	VERY LOW
Holotranscobalamin <21 pmol/L for diagnosing metabolic deficiency (MMA >0.45 µmol/)L							
1 diagnostic accuracy observational cohort study	360	Serious[7]	Not serious	Very serious[2]	Serious[5]	Sensitivity=0.64 (0.49-0.77)	VERY LOW
		Serious[7]	Not serious	Very serious[2]	Not serious	Specificity=0.88 (0.84-0.91)	VERY LOW
Holotranscobalamin <32 pmol/L for diagnosing metabolic deficiency (MMA >0.45 µmol/)L							
1 diagnostic accuracy observational cohort study	360	Serious[7]	Not serious	Very serious[2]	Serious[3]	Sensitivity=0.83 (0.69-0.92)	VERY LOW
		Serious[7]	Not serious	Very serious[2]	Not serious	Specificity=0.60 (0.54-0.66)	VERY LOW
Holotranscobalamin <35 pM for diagnosing deficiency (MMA >300 nM)							
1 diagnosti	1359	Serious[8]	Not serious	Very serious[2]	Not serious	Sensitivity=0.72 (0.67-0.76)	VERY LOW

Studies	N	Risk of bias	Inconsistency	Indirectness	Imprecision	Effect size (95%CI)	Quality
c accuracy observational cohort study		Serious[8]	Not serious	Very serious[2]	Not serious	Specificity=0.54 (0.51-0.57)	VERY LOW
Holotranscobalamin <22 pM for diagnosing deficiency (MMA >300 nM)							
1 diagnostic accuracy observational cohort study	1034	Serious[8]	Not serious	Very serious[2]	Serious[3]	Sensitivity=0.90 (0.85-0.94)	VERY LOW
		Serious[8]	Not serious	Very serious[2]	Not serious	Specificity=0.27 (0.24-0.30)	VERY LOW
Holotranscobalamin <76 pM for diagnosing deficiency (MMA >300 nM)							
1 diagnostic accuracy observational cohort study	1034	Serious[8]	Not serious	Very serious[2]	Not serious	Sensitivity=0.21 (0.15-0.27)	VERY LOW
		Serious[8]	Not serious	Very serious[2]	Not serious	Specificity=0.90 (0.88-0.92)	VERY LOW
Holotranscobalamin <19 pmol/l for diagnosing metabolic deficiency (MMA >47 µg/)l (normal renal function)							
1 diagnostic accuracy retrospective observational cohort study	125	Very serious[9]	Not serious	Very serious[2]	Not serious	Sensitivity=0.06 (0.01-0.30)	VERY LOW
		Very serious[9]	Not serious	Very serious[2]	Not serious	Specificity=0.97 (0.92-0.99)	VERY LOW
Holotranscobalamin <42 pmol/l for diagnosing metabolic deficiency (MMA >47 µg/)l (normal renal function)							
1 diagnostic accuracy retrospective observational cohort study	125	Very serious[9]	Not serious	Very serious[2]	Serious[5]	Sensitivity=0.56 (0.30-0.80)	VERY LOW
		Very serious[9]	Not serious	Very serious[2]	Not serious	Specificity=0.50 (0.41-0.60)	VERY LOW
Holotranscobalamin <67 pmol/l for diagnosing metabolic deficiency (MMA >47 µg/)l (normal renal function)							
1 diagnostic accuracy retrospective	125	Very serious[9]	Not serious	Very serious[2]	Serious[3]	Sensitivity=0.88 (0.62-0.98)	VERY LOW
		Very serious[9]	Not serious	Very serious[2]	Not serious	Specificity=0.14 (0.08-0.22)	VERY LOW

Studies	N	Risk of bias	Inconsistency	Indirectness	Imprecision	Effect size (95%CI)	Quality
observational cohort study							
Holotranscobalamin <19 pmol/l for diagnosing metabolic deficiency (MMA >47 µg/)l (whole cohort)							
1 diagnostic accuracy retrospective observational cohort study	171	Very serious[9]	Not serious	Very serious[2]	Not serious	Sensitivity=0.04 (0.01-0.21)	VERY LOW
		Very serious[9]	Not serious	Very serious[2]	Not serious	Specificity=0.97 (0.93-0.99)	VERY LOW
Holotranscobalamin <42 pmol/l for diagnosing metabolic deficiency (MMA >47 µg/)l (whole cohort)							
1 diagnostic accuracy retrospective observational cohort study	171	Very serious[9]	Not serious	Very serious[2]	Serious[5]	Sensitivity=0.46 (0.26-0.67)	VERY LOW
		Very serious[9]	Not serious	Very serious[2]	Not serious	Specificity=0.53 (0.45-0.62)	VERY LOW
Holotranscobalamin <77 pmol/l for diagnosing metabolic deficiency (MMA >47 µg/)l (whole cohort)							
1 diagnostic accuracy retrospective observational cohort study	171	Very serious[9]	Not serious	Very serious[2]	Serious[3]	Sensitivity=0.96 (0.79-1.00)	VERY LOW
		Very serious[9]	Not serious	Very serious[2]	Not serious	Specificity=0.09 (0.05-0.15)	VERY LOW

[1] *Very serious risk of bias due to lack of reporting on patient selection, details of the index tests, whether index tests and reference standard were conducted and interpreted without knowledge of each other and the time interval between their measurement.*

[2] *Evidence was downgraded by 1 increment if the majority of studies included an indirect population, and by 2 increments if the majority of studies included a very indirect population.*

[3] *Confidence interval crossed the decision threshold corresponding to 'high sensitivity' (90%).*

[4] *Confidence interval crossed the decision threshold corresponding to 'high specificity' (70%).*

[5] *Confidence interval crossed the decision threshold corresponding to 'low sensitivity' (60%).*

[6] *Serious risk of bias due to patient selection bias (analysis based on samples rather than patients).*

[7] *Serious risk of bias due to unclear method of patient selection and lack of reporting as to whether index tests and reference standard were conducted and interpreted without knowledge of each other.*

[8] *Serious due to lack of reporting on methods of patient selection, patient characteristics and whether index tests and reference standard were conducted and interpreted without knowledge of each other.*

[9] *Very serious due to lack of reporting regarding whether index tests and reference standard were conducted and interpreted without knowledge of each other and the time interval between index tests and reference standard and high number of participants excluded from the analysis with little explanation.*

Table 5: Clinical evidence summary: holotranscobalamin (second line)

Studies	N	Risk of bias	Inconsistency	Indirectness	Imprecision	Effect size (95%CI)	Quality
Holotranscobalamin <38 pmol/L for diagnosing response to treatment							
1 diagnostic accuracy observational cohort study	27	Serious[1]	Not serious	Very serious[2]	Serious[3]	Sensitivity=0.84 (0.74-0.92)	VERY LOW
		Serious[1]	Not serious	Very serious[2]	Not serious	Specificity=0.76 (0.68-0.83)	VERY LOW

[1] *Risk of bias was assessed using the QUADAS-2 checklist. The evidence was downgraded by 1 increment if the majority of studies were rated at high risk of bias, and downgraded by 2 increments if the majority of studies were rated at very high risk of bias.*

[2] *Evidence was downgraded by 1 increment if the majority of studies included an indirect population, and by 2 increments if the majority of studies included a very indirect population.*

[3] *Confidence interval crossed the decision threshold corresponding to 'high sensitivity' (80%).*

Table 6: Clinical evidence summary: methylmalonic acid (first line)

Studies	N	Risk of bias	Inconsistency	Indirectness	Imprecision	Effect size (95%CI)	Quality
MMA >466 nmol/L for diagnosing possible/probable deficiency (4cB12 ≤ -1.5)							
1 diagnostic accuracy retrospective observational cohort study	11833	Serious[1]	Not serious	Very serious[2]	Serious[3]	Sensitivity=0.95 (0.86-0.99)	VERY LOW
		Serious[1]	Not serious	Very serious[2]	Not serious	Specificity=0.96 (0.96-0.97)	VERY LOW
MMA >158 nmol/L for diagnosing possible/probable deficiency (4cB12 ≤ -1.5)							
1 diagnostic accuracy retrospective observational cohort study	11833	Serious[1]	Not serious	Very serious[2]	Not serious	Sensitivity=0.98 (0.91-1.00)	VERY LOW
		Serious[1]	Not serious	Very serious[2]	Not serious	Specificity=0.39 (0.38-0.40)	VERY LOW
MMA >723 nmol/L for diagnosing possible/probable deficiency (4cB12 ≤ -1.5)							
1 diagnostic accuracy retrospective observational	11833	Serious[1]	Not serious	Very serious[2]	Serious[4]	Sensitivity=0.72 (0.59-0.83)	VERY LOW
		Serious[1]	Not serious	Very serious[2]	Not serious	Specificity=0.99 (0.99-0.99)	VERY LOW

Studies	N	Risk of bias	Inconsistency	Indirectness	Imprecision	Effect size (95%CI)	Quality
cohort study							
MMA >245 nmol/L for diagnosing subclinical deficiency (4cB12 ≤ -0.5 and >-1.5)							
1 diagnostic accuracy retrospective observational cohort study	1183 3	Serious[1]	Not serious	Very serious[2]	Not serious	Sensitivity=0.82 (0.79-0.84)	VERY LOW
		Serious[1]	Not serious	Very serious[2]	Not serious	Specificity=0.83 (0.83-0.84)	VERY LOW
MMA >152 nmol/L for diagnosing subclinical deficiency (4cB12 ≤ -0.5 and >-1.5)							
1 diagnostic accuracy retrospective observational cohort study	1183 3	Serious[1]	Not serious	Very serious[2]	Not serious	Sensitivity=0.99 (0.98-1.00)	VERY LOW
		Serious[1]	Not serious	Very serious[2]	Not serious	Specificity=0.38 (0.37-0.39)	VERY LOW
MMA >480 nmol/L for diagnosing subclinical deficiency (4cB12 ≤ -0.5 and >-1.5)							
1 diagnostic accuracy retrospective observational cohort study	1183 3	Serious[1]	Not serious	Very serious[2]	Not serious	Sensitivity=0.29 (0.26-0.32)	VERY LOW
		Serious[1]	Not serious	Very serious[2]	Not serious	Specificity=0.99 (0.99-0.99)	VERY LOW
Urinary MMA >5 µg/mg creatinine for diagnosing clinical deficiency (all abnormalities suggesting deficiency, or fewer if lessened in response to treatment)							
1 diagnostic accuracy prospective observational cohort study	96	Serious[5]	Not serious	Very serious[2]	Very serious[6]	Sensitivity=1.00 (0.59-1.00)	VERY LOW
		Serious[5]	Not serious	Very serious[2]	Not serious	Specificity=0.99 (0.94-1.00)	VERY LOW

[1] *Risk of bias was assessed using the QUADAS-2 checklist. The evidence was downgraded by 1 increment if the majority of studies were rated at high risk of bias, and downgraded by 2 increments if the majority of studies were rated at very high risk of bias.*

[2] *Evidence was downgraded by 1 increment if the majority of studies included an indirect population, and by 2 increments if the majority of studies included a very indirect population.*

[3] *Confidence interval crossed the decision threshold corresponding to 'high sensitivity' (90%).*

[4] *Confidence interval crossed the decision threshold corresponding to 'low sensitivity' (60%).*

[5] *Serious risk of bias due to time interval between index test and reference standard.*

[6] *Confidence interval crossed both decision thresholds corresponding to 'high sensitivity' (90%) and low sensitivity' (60%).*

Table 7: Clinical evidence summary: methylmalonic acid (second line)

Studies	N	Risk of bias	Inconsistency	Indirectness	Imprecision	Effect size (95%CI)	Quality
MMA >0.47 µmol/L for diagnosing response to treatment							
1 diagnostic accuracy observational cohort study	27	Serious[1]	Not serious	Very serious[2]	Very serious[3]	Sensitivity=0.73 (0.45-0.92)	VERY LOW
		Serious[1]	Not serious	Very serious[2]	Serious[4]	Specificity=0.67 (0.35-0.90)	VERY LOW
MMA >0.34 µmol l-1 for diagnosing clinical deficiency (cobalamin <100 pmol l-1 and abnormal Schilling test and/or megaloblastic bone marrow morphology)							
1 diagnostic accuracy prospective observational cohort study	42	Serious[5]	Not serious	Very serious[2]	Not serious	Sensitivity=0.97 (0.83-1.00)	VERY LOW
		Serious[5]	Not serious	Very serious[2]	Very serious[3]	Specificity=0.91 (0.59-1.00)	VERY LOW

[1] *Serious risk of bias due to lack of reporting as to whether index tests and reference standard were conducted and interpreted without knowledge of each other and the 3-month time interval between their measurement.*

[2] *Evidence was downgraded by 1 increment if the majority of studies included an indirect population, and by 2 increments if the majority of studies included a very indirect population.*

[3] *Confidence interval crossed the decision threshold corresponding to 'high sensitivity' (90%).*

[4] *Confidence interval crossed both decision thresholds corresponding to 'high specificity' (90%) and low specificity' (60%).*

[5] *Serious risk of bias due to unclear time interval between index test and reference standard.*

Table 8: Clinical evidence summary: homocysteine (first line)

Studies	N	Risk of bias	Inconsistency	Indirectness	Imprecision	Effect size (95%CI)	Quality
Plasma total homocysteine >15 µmol/L for diagnosing deficiency (MMA response to treatment)							
1 diagnostic accuracy observational cohort study	187	Serious[1]	Not serious	Very serious[2]	Serious[3]	Sensitivity=0.73 (0.58-0.84)	VERY LOW
		Serious[1]	Not serious	Very serious[2]	Serious[4]	Specificity=0.68 (0.59-0.75)	VERY LOW
Plasma total homocysteine >11.3 µmol/L for diagnosing deficiency (MMA response to treatment)							
1 diagnosti	187	Serious[1]	Not serious	Very serious[2]	Serious[5]	Sensitivity=0.90 (0.79-0.97)	VERY LOW

Studies	N	Risk of bias	Inconsistency	Indirectness	Imprecision	Effect size (95%CI)	Quality
c accuracy prospective observational cohort study		Serious[1]	Not serious	Very serious[2]	Serious[6]	Specificity=0.38 (0.30-0.47)	VERY LOW
Homocysteine for diagnosing B12 deficiency (serum B12 <200 pg/mL)							
1 diagnostic accuracy observational cohort study	217	Very serious[7]	Not serious	Very serious[2]	Not serious	Sensitivity=0.73 (0.61-0.83)	VERY LOW
		Very serious[7]	Not serious	Very serious[2]	Not serious	Specificity=0.80 (0.73-0.86)	VERY LOW
Homocysteine for diagnosing B12 deficiency and borderline deficiency (serum B12 ≤350 pg/mL)							
1 diagnostic accuracy observational cohort study	217	Very serious[7]	Not serious	Very serious[2]	Not serious	Sensitivity=0.46 (0.39-0.54)	VERY LOW
		Very serious[7]	Not serious	Very serious[2]	Not serious	Specificity=1.00 (0.92-1.00)	VERY LOW
Homocysteine >16.4 µmol/L for diagnosing possible/probable deficiency (4cB12 ≤ -1.5)							
1 diagnostic accuracy retrospective observational cohort study	1183 3	Serious[8]	Not serious	Very serious[2]	Serious[5]	Sensitivity=0.88 (0.77-0.95)	VERY LOW
		Serious[8]	Not serious	Very serious[2]	Not serious	Specificity=0.81 (0.80-0.82)	VERY LOW
Homocysteine >6.2 µmol/L for diagnosing possible/probable deficiency (4cB12 ≤ -1.5)							
1 diagnostic accuracy retrospective observational cohort study	1183 3	Serious[8]	Not serious	Very serious[2]	Not serious	Sensitivity=0.98 (0.91-1.00)	VERY LOW
		Serious[8]	Not serious	Very serious[2]	Not serious	Specificity=0.03 (0.03-0.03)	VERY LOW
Homocysteine >34 µmol/L for diagnosing possible/probable deficiency (4cB12 ≤ -1.5)							
1 diagnostic accuracy retrospective	1183 3	Serious[8]	Not serious	Very serious[2]	Not serious	Sensitivity=0.33 (0.21-0.46)	VERY LOW
		Serious[8]	Not serious	Very serious[2]	Not serious	Specificity=0.99 (0.99-0.99)	VERY LOW

Studies	N	Risk of bias	Inconsistency	Indirectness	Imprecision	Effect size (95%CI)	Quality
observational cohort study							
Homocysteine >15 µmol/L for diagnosing subclinical deficiency (4cB12 ≤ -0.5 and >-1.5)							
1 diagnostic accuracy retrospective observational cohort study	1183 3	Serious[8]	Not serious	Very serious[2]	Serious[3]	Sensitivity=0.68 (0.65-0.71)	VERY LOW
		Serious[8]	Not serious	Very serious[2]	Not serious	Specificity=0.77 (0.76-0.77)	VERY LOW
Homocysteine >8 µmol/L for diagnosing subclinical deficiency (4cB12 ≤ -0.5 and >-1.5)							
1 diagnostic accuracy retrospective observational cohort study	1183 3	Serious[8]	Not serious	Very serious[2]	Not serious	Sensitivity=0.99 (0.98-1.00)	VERY LOW
		Serious[8]	Not serious	Very serious[2]	Not serious	Specificity=0.13 (0.12-0.13)	VERY LOW
Homocysteine >29 µmol/L for diagnosing subclinical deficiency (4cB12 ≤ -0.5 and >-1.5)							
1 diagnostic accuracy retrospective observational cohort study	1183 3	Serious[8]	Not serious	Very serious[2]	Not serious	Sensitivity=0.11 (0.10-0.14)	VERY LOW
		Serious[8]	Not serious	Very serious[2]	Not serious	Specificity=0.99 (0.99-0.99)	VERY LOW

[1] Serious risk of bias due to lack of reporting as to whether index tests and reference standard were conducted and interpreted without knowledge of each other and the time interval between their measurement.

[2] Evidence was downgraded by 1 increment if the majority of studies included an indirect population, and by 2 increments if the majority of studies included a very indirect population.

[3] Confidence interval crossed the decision threshold corresponding to ''low sensitivity' (60%).

[4] Confidence interval crossed the decision threshold corresponding to 'high specificity' (70%).

[5] Confidence interval crossed the decision threshold corresponding to ''high sensitivity' (90%).

[6] Confidence interval crossed the decision threshold corresponding to 'low specificity' (40%).

[7] Very serious risk of bias due to lack of reporting on patient selection, details of the index tests, whether index tests and reference standard were conducted and interpreted without knowledge of each other and the time interval between their measurement.

[8] Serious risk of bias due to patient selection bias (analysis based on samples rather than patients).

Table 9: Clinical evidence summary: homocysteine (second line)

Studies	N	Risk of bias	Inconsistency	Indirectness	Imprecision	Effect size (95%CI)	Quality
Homocysteine >15 µmol/L for diagnosing response to treatment							
1 diagnostic accuracy observational cohort study	27	Serious[1]	Not serious	Very serious[2]	Serious[3]	Sensitivity=1.00 (0.78-1.00)	VERY LOW
		Serious[1]	Not serious	Very serious[2]	Very serious[4]	Specificity=0.42 (0.15-0.72)	VERY LOW

[1] *Serious risk of bias due to lack of reporting as to whether index tests and reference standard were conducted and interpreted without knowledge of each other and the 3-month time interval between their measurement.*

[2] *Evidence was downgraded by 1 increment if the majority of studies included an indirect population, and by 2 increments if the majority of studies included a very indirect population.*

[3] *Confidence interval crossed the decision threshold corresponding to ''high sensitivity' (80%).*

[4] *Confidence interval crossed the decision threshold corresponding to 'low specificity' (60%).*

Table 10: Clinical evidence summary: combinations (first line)

Studies	N	Risk of bias	Inconsistency	Indirectness	Imprecision	Effect size (95%CI)	Quality
2cB12HoloTC/MMA for diagnosing inadequate B12 status (4cB12 <-0.5)							
1 diagnostic accuracy retrospective observational cohort study	3614	Serious[1]	Not serious	Very serious[2]	Not serious	Sensitivity=0.76 (0.71-0.81)	VERY LOW
		Serious[1]	Not serious	Very serious[2]	Not serious	Specificity=0.98 (0.97-0.98)	VERY LOW
2cB12B12/MMA for diagnosing inadequate B12 status (4cB12 <-0.5)							
1 diagnostic accuracy retrospective observational cohort study	3614	Serious[1]	Not serious	Very serious[2]	Serious[3]	Sensitivity=0.56 (0.50-0.62)	VERY LOW
		Serious[1]	Not serious	Very serious[2]	Not serious	Specificity=0.99 (0.99-0.99)	VERY LOW
2cB12B12/Hcy for diagnosing inadequate B12 status (4cB12 <-0.5)							
1 diagnostic accuracy retrospective observational cohort study	3614	Serious[1]	Not serious	Very serious[2]	Not serious	Sensitivity=0.68 (0.62-0.73)	VERY LOW
		Serious[1]	Not serious	Very serious[2]	Not serious	Specificity=0.95 (0.94-0.96)	VERY LOW

Studies	N	Risk of bias	Inconsistency	Indirectness	Imprecision	Effect size (95%CI)	Quality
2cB12HoloTC/B12 for diagnosing inadequate B12 status (4cB12 <-0.5)							
1 diagnostic accuracy retrospective observational cohort study	3614	Serious[1]	Not serious	Very serious[2]	Not serious	Sensitivity=0.79 (0.74-0.83)	VERY LOW
		Serious[1]	Not serious	Very serious[2]	Not serious	Specificity=0.96 (0.95-0.97)	VERY LOW
2cB12HoloTC/Hcy for diagnosing inadequate B12 status (4cB12 <-0.5)							
1 diagnostic accuracy retrospective observational cohort study	3614	Serious[1]	Not serious	Very serious[2]	Serious[4]	Sensitivity=0.92 (0.88-0.95)	VERY LOW
		Serious[1]	Not serious	Very serious[2]	Not serious	Specificity=0.90 (0.89-0.91)	VERY LOW
2cB12MMA/Hcy for diagnosing inadequate B12 status (4cB12 <-0.5)							
1 diagnostic accuracy retrospective observational cohort study	3614	Serious[1]	Not serious	Very serious[2]	Not serious	Sensitivity=0.67 (0.61-0.72)	VERY LOW
		Serious[1]	Not serious	Very serious[2]	Not serious	Specificity=0.95 (0.94-0.96)	VERY LOW
3cB12HoloTC/B12/MMA for diagnosing inadequate B12 status (4cB12 <-0.5)							
1 diagnostic accuracy retrospective observational cohort study	3614	Serious[1]	Not serious	Very serious[2]	Not serious	Sensitivity=0.78 (0.73-0.82)	VERY LOW
		Serious[1]	Not serious	Very serious[2]	Not serious	Specificity=0.99 (0.99-0.99)	VERY LOW
3cB12MMA/HoloTC/Hcy for diagnosing inadequate B12 status (4cB12 <-0.5)							
1 diagnostic accuracy retrospective observational cohort study	3614	Serious[1]	Not serious	Very serious[2]	Serious[4]	Sensitivity=0.87 (0.83-0.91)	VERY LOW
		Serious[1]	Not serious	Very serious[2]	Not serious	Specificity=0.98 (0.97-0.98)	VERY LOW
3cB12HoloTC/B12/Hcy for diagnosing inadequate B12 status (4cB12 <-0.5)							

Studies	N	Risk of bias	Inconsistency	Indirectness	Imprecision	Effect size (95%CI)	Quality
1 diagnostic accuracy retrospective observational cohort study	3614	Serious[1]	Not serious	Very serious[2]	Serious[4]	Sensitivity=0.87 (0.83-0.91)	VERY LOW
		Serious[1]	Not serious	Very serious[2]	Not serious	Specificity=0.96 (0.95-0.97)	VERY LOW
3cB12MMA/B12/Hcy for diagnosing inadequate B12 status (4cB12 <-0.5)							
1 diagnostic accuracy retrospective observational cohort study	3614	Serious[1]	Not serious	Very serious[2]	Not serious	Sensitivity=0.67 (0.61-0.72)	VERY LOW
		Serious[1]	Not serious	Very serious[2]	Not serious	Specificity=0.99 (0.99-0.99)	VERY LOW

[1] *Serious risk of bias due to patient selection bias (analysis based on samples rather than patients).*

[2] *Evidence was downgraded by 1 increment if the majority of studies included an indirect population, and by 2 increments if the majority of studies included a very indirect population.*

[3] *Confidence interval crossed the decision threshold corresponding to ''low sensitivity' (60%).*

[4] *Confidence interval crossed the decision threshold corresponding to 'high sensitivity' (90%).*

Table 11: Clinical evidence summary: combinations (first and second line)

Studies	N	Risk of bias	Inconsistency	Indirectness	Imprecision	Effect size (95%CI)	Quality
Harrington's algorithm for diagnosing inadequate B12 status (4cB12 <-0.5)							
1 diagnostic accuracy retrospective observational cohort study	3614	Serious[1]	Not serious	Very serious[2]	Not serious	Sensitivity=0.83 (0.78-0.87)	VERY LOW
		Serious[1]	Not serious	Very serious[2]	Not serious	Specificity=0.93 (0.92-0.94)	VERY LOW

[1] *Serious risk of bias due to patient selection bias (analysis based on samples rather than patients).*

[2] *Evidence was downgraded by 1 increment if the majority of studies included an indirect population, and by 2 increments if the majority of studies included a very indirect population.*

1.1.7 Economic evidence

There was no economic evidence identified relevant to the review question.

1.2 Review question

What are the most clinically and cost-effective ways to diagnose vitamin B12 deficiency, including the serum cobalamin assay and holotranscobalamin, methylmalonic acid and homocysteine tests?

1.2.1 Summary of the protocol

For full details see the review protocol in Appendix A.

Table 12: PICO characteristics of review question

Population	Inclusion: Adults with suspected vitamin B12 deficiency. Exclusion: None Stratified by: • Age (adults 16/18 years and older; older adults 65 years and older) • Third trimester of pregnancy (third trimester; first two trimesters and not pregnant) • Ethnicity (Afro-Caribbean; other) • Sex (male; female) (study defined) for Homocysteine test only
Interventions	The following as stand-alone tests or in combination: • Serum cobalamin assay • Holotranscobalamin test • Methylmalonic acid test (including urinary) • Homocysteine test Treatment as a result of a positive test: • Vitamin B12 replacement o Hydroxocobalamin o Cyanocobalamin o Cobalamin/B12 Strata: reference ranges as defined by the studies
Comparisons	• All tests and combinations of tests compared with each other • No test (treatment only)
Outcomes	• Randomised controlled trials • Systematic reviews of RCTs
Study design	All outcomes are considered equally important for decision making and therefore have all been rated as critical: • quality of life (such as EQ5D, SF36) • patient-reported outcomes (PROM scores including some/all symptoms): o fatigue o sleep o peripheral neuropathy o cognition o psychiatric symptoms o pain • haematological values • complications and adverse events o mortality o bleeds

	o self-harm o nerve damage o frailty/falls o severe cognitive effects o postural hypotension • patient concern around unexpected lab results (health anxiety score) • incorrect/delayed diagnosis • inappropriate additional tests • adherence to treatment • education/work absence Time point: any time point available

1.2.2 Methods and process

This evidence review was developed using the methods and process described in Developing NICE guidelines: the manual. Methods specific to this review question are described in the review protocol in appendix A and the methods document.

Declarations of interest were recorded according to NICE's conflicts of interest policy.

1.2.3 Effectiveness evidence

1.2.3.1 Included studies

No relevant clinical studies comparing serum cobalamin assay, holotranscobalamin, methylmalonic acid, homocysteine, or a combination of tests with each other or with treatment only were identified.

See the study selection flow chart in Appendix C.

1.2.4 Summary of studies included in the effectiveness evidence

No studies were included.

1.2.5 Summary of the effectiveness evidence

No evidence was identified.

1.2.6 Economic evidence

1.2.6.1 Included studies

One health economic study with the relevant comparison was included in this review. [12] This is summarised in the health economic evidence profile below (Table 13) and the health economic evidence table in Appendix H.

1.2.6.2 Excluded studies

No relevant health economic studies were excluded due to assessment of limited applicability or methodological limitations.

See also the health economic study selection flow chart in Appendix G.

1.2.7 Summary of included economic evidence

Table 13: Health economic evidence profile: Do not test and treat (with IM or oral supplement) vs test and treat (with IM or oral supplement)

Study	Applic-ability	Limitations	Other comments	Incremental cost	Incremental effects	Cost effectiveness	Uncertainty
Mnatzaganian 2015[12] ([Australia])	Partially applicable[(a)]	Potentially serious limitations[(b)]	• Decision tree model comparing five mutually exclusive diagnostic-therapeutic strategies using serum cobalamin as diagnostic test. • Cost-utility analysis (QALYs) • Population: 18 years of age or older - presenting with fatigue • Comparators: 1. Do not test and do not treat 2. Serum test and treat with IM B12 3. Serum test and treat with oral B12 supplement 4. Do not test and treat all with IM B12 5. Do not test and treat all with oral B12 supplement Time horizon: 3 months.	2-1: £71[(c)] 3-2: -£23[(c)] 4-3: £108[(c)] 5-4: £94[(c)]	2-1: 0.01 3-2: 0 4-3: 0.01 5-4: 0	Intervention 5 dominates 2 and 4 and extendedly dominates 3 Intervention 5 vs intervention 1: £3,105 per QALY	Probability intervention 5 – "do not test but treat all with oral supplements" cost effective (£20/£30K threshold): 100% A probabilistic analysis was performed which included sensitivity and specificity of the diagnostic test, cost, and utility input parameter values. Further deterministic sensitivity analysis was done with varying the prevalence levels of cobalamin deficiency at 1%, 5% 10% and 20%. Results remained robust in all analyses.

Abbreviations: ICER= incremental cost-effectiveness ratio; IM = intramuscular, QALY= quality-adjusted life years

(a) The UK tariff was not used for EQ-5D; The utility scores were derived using hypothetical state scenarios which may not be comparable to one obtained by using patient data. Study is Australia based.

(b) This study does not compare the different available diagnostic tests for b12 deficiency and focuses on diagnosis and intervention together using serum cobalamin testing with oral and IM treatment. Fatigue is only one symptom which may be related to B12 deficiency, so this study does not capture all potential B12 deficient patients. Only the first consultation was considered; all screening tests that could have been ordered—other than serum cobalamin—were not included in this economic evaluation. The cost of misdiagnosis/referral to specialists was not included which could have a significant impact of the cost-effectiveness. There is uncertainty regarding the baseline prevalence of B12 deficiency. Risk of recurrence of deficiency or symptoms after three months were not explored.

(c) [2013] costs/2013 USA dollars converted to UK pounds[18]. Cost components incorporated: GP-patient consultation fee, serum cobalamin test, Patient Episode Initiation (specimen) fees, medication costs, service costs for IM injections.

1.2.8 Economic model

1.2.8.1 Model specification

Population: People who have had an indeterminate result with either an active B12 test or total B12 test.

Comparison: 'Methylmalonic Acid (MMA) testing, treat positive' vs 'No MMA testing, no treatment'.

Perspective: National Health Service and Personal Social Services.

Outcomes: Quality-adjusted life-years (QALYs).

Details of this model can be found in **Error! Reference source not found.**.

1.2.8.2 Model results

The cost per QALY gained from 'MMA testing, treat positive' vs 'no MMA testing, no treatment' was less than £20,000 in the base case analysis (Table 14).

Table 14: Health economic evidence profile: MMA testing vs No MMA testing

Study	Applicability	Limitations	Other comments	Incremental cost	Incremental effects	Cost effectiveness	Uncertainty
NICE Methods and Economics Team 2023	Directly applicable	Potentially serious limitations[(a)]	• Decision tree model • Comparators: 1.MMA testing, treating positive. 2.No MMA testing, no treatment. • Cost-utility analysis (QALYs) • Population: People that have had an indeterminate first line	£29.62	0.0075 QALYs	£3,946 per QALY	The model was subject to various deterministic analyses. The cost of MMA testing, underlying prevalence of elevated MMA in the population and the time horizon of treatment were analysed. When comparing MMA testing vs 'no MMA testing, no treatment', the ICER was always below £11,500

Study	Applicability	Limitations	Other comments	Incremental cost	Incremental effects	Cost effectiveness	Uncertainty
			test result (either active B12 or total B12). Time horizon: 3 months				thus indicating MMA is cost effective.

Abbreviations: ICER= incremental cost-effectiveness ratio; MMA= Methylmalonic Acid; QALY= quality-adjusted life years.

(a) Underlying prevalence taken from one study, diagnostic pathway assumed, for strategy 2, there is uncertainty in the diagnostic and clinical pathway if a B12 deficient person is not tested, cyanocobalamin dose assumed to be 1mg/daily, MMA testing assumed to be 100% accurate, utility data taken from Australian study based on expert opinion. Due to limited data no probabilistic sensitivity analysis.

(b) 2022/23 UK pounds. Cost components incorporated: Oral treatment cost (Orobalin 1mg/daily), MMA test cost, One GP appointment for people in strategy 2 that are B12 deficient.

Sensitivity analyses (see Table 15) using varying underlying elevated MMA prevalence's, MMA cost and time horizons of treatments were explored to check how likely it was for the introduction of MMA to be cost effective. The prevalence's of elevated MMA were based on the reported highest and lowest and mean reported in Sobczyńska-Malefora et al. (2015); these were 14% (for active B12 25-29 pmol/L),24.3% (mean of active B12 25-70 pmol/L) and 40% (for active B12 65-70 pmol/L). The costs of MMA were obtained from the committee members with a range of £11 - £49.41 with a mean of £30.35. The time horizon was varied at three, four, five, six and twelve months with the assumption that the treatment will continue over this period.

Table 15: Sensitivity analyses (deterministic) – Incremental cost per QALY, MMA testing vs No MMA testing, no treatment

Time horizon	High MMA cost, low prevalence	High MMA cost, average prevalence	High MMA cost, high prevalence	Average MMA cost, low prevalence	Average MMA cost, average prevalence	Average MMA cost, high prevalence	Low MMA cost, low prevalence	Low MMA cost, average prevalence	Low MMA cost, high prevalence
3 months	£11,333	£6,484	£3,903	£6,924	£3,946*	£2,360	£2,447	£1,367	£793
4 months	£6,337	£3,701	£2,298	£3,940	£2,321	£1,459	£1,506	£919	£607
5 months	£4,472	£2,662	£1,698	£2,826	£1,714	£1,122	£1,155	£752	£537
6 months	£3,497	£2,119	£1,385	£2,244	£1,397	£947	£971	£665	£501
12 months	£1,665	£1,098	£797	£1,150	£802	£616	£626	£500	£433

* Base case

An alternative strategy of ‘no MMA testing, treating all’ was the subject of sensitivity analyses (see Appendix I). When comparing MMA testing to ‘no MMA testing, treat all’, there was an extra cost of £7 per person if treatment was for 3 months. But with longer treatment periods testing was cost saving. In cases where the MMA cost was low, MMA testing was cost saving regardless of the prevalence and treatment duration.

1.2.9 Unit costs

Relevant unit costs are provided below to aid consideration of cost effectiveness.

Table 16: Test costs

Resource	Unit costs	Source
Total B12 (cyanocobalamin)	£2.20	Obtained from committee members (average)
Active B12 (holotranscobalamin)	£18.50	Obtained from committee members (average)
MMA (methylmalonic acid)	£30.40	Obtained from committee members (average)
Homocysteine	£35.70	Obtained from committee members (average)

1.2.10 Evidence statements

Economic

- One published cost–utility analysis found that total B12 cyanocobalamin testing compared to not testing total B12 was more costly without additional health benefit as a first line test. This analysis was assessed as partially applicable with potentially serious limitations.

- One original cost-utility analysis found that MMA testing was cost effective compared to no test for people who have an indeterminate first line test result (ICER: £3,946).

1.3 Committee discussion

The committee discussion of the review on the clinical and cost effectiveness of tests for diagnosing vitamin B12 deficiency is included in the discussion of the review on the diagnostic accuracy of tests for vitamin B12 deficiency.

1.3.1 The outcomes that matter most

Diagnostic test accuracy

Diagnostic accuracy for vitamin B12 deficiency was the outcome prioritised for this review. The Guideline Committee considered sensitivity the most important measure for first line tests to minimise the risk of false negative results. False negative results would mean that people with vitamin B12 deficiency would not receive appropriate treatment, which could lead to worsening symptoms and further complications. Specificity was considered the most important measure for second line tests to guard against a potentially high number of false positive results from the first line test. Although treatment is non-toxic, a diagnosis of vitamin B12 deficiency can have lifelong implications and if a person is misdiagnosed, this could delay or prevent an alternative diagnosis being identified.

Diagnostic test and treat

Quality of life, patient reported outcomes (including fatigue, sleep, peripheral neuropathy, cognition, psychiatric symptoms and pain), haematological values, complications and adverse events, patient concerns around unexpected laboratory results, incorrect/delayed diagnosis, inappropriate additional tests, adherence to treatment and education/work

absence were considered by the Guideline Committee to be equally important for decision making and were therefore all rated as critical. Most of the examples of potential adverse events prespecified by the committee were related to possible consequences of untreated vitamin B12 deficiency, rather than the testing and treatment strategies. No evidence was identified for any of the outcomes.

1.3.2 The quality of the evidence

Diagnostic test accuracy

Evidence was identified for total B12 (cobalamin) as a first line test, active B12 (holotranscobalamin) as a first line test and second line test, methylmalonic acid (MMA) as a first line and second line test and homocysteine as a first line and second line test, for combinations of tests as first line tests and for active B12 and MMA as staged tests. No evidence was identified for women and people in the third trimester of pregnancy, Afro-Caribbean ethnicity, or males/females (homocysteine test).

Several different reference ranges and cut-offs for index test positivity were reported in the studies. Out of 10 studies included, no 2 studies used the same reference standard for defining deficiency. Reference standards included MMA/haematological response to treatment, total B12 concentration below the reference range, MMA concentration below the reference range, combinations of deficiency marker values and clinical abnormalities suggestive of deficiency. Some studies used MMA concentration as an index test, whereas others used it as the reference standard, making it difficult to triangulate the results across the studies.

Evidence was of very low quality, mainly due to risk of bias and indirectness. Most studies were at serious or very serious risk of bias, mainly due to issues with patient selection, lack of reporting on conduct/interpretation of the index and reference tests and the time interval between them.

All evidence was considered very indirect due to the lack of a gold standard test for vitamin B12 deficiency, the lack of study reporting on use of vitamin B12 containing supplements and mixed/unclear strata. All evidence was in mixed adult populations, including adults (≥16/18 years) and older adults (≥65 years), males and females. Ethnicity and pregnancy status was not reported.

Some outcomes were downgraded for serious or very serious imprecision, depending on whether the confidence intervals crossed one or both clinical decision thresholds. No meta-analysis was performed due to the different index test cut-offs and reference standards used; therefore, inconsistency was not relevant for this review.

The committee considered the limitations of the evidence outlined above and acknowledged the difficulty in drawing any firm conclusions.

Diagnostic test and treat

No evidence was identified for the clinical and cost effectiveness of different test and treat strategies.

1.3.3 Benefits and harms

Ranges of sensitivities and specificities for the tests recommended by the committee:

The sensitivities and specificities of the index tests varied widely within and between studies, depending on the different reference ranges/cut-offs and reference standards used, but no clear pattern emerged. The committee considered the very low quality and the limitations of the diagnostic test accuracy evidence together with the absence of clinical and cost

effectiveness evidence and determined that there was insufficient evidence upon which to base recommendations. The committee agreed that the weak evidence base reflected the difficulties in diagnosis and lack of a single reliable diagnostic test, including the most widely used total B12 assay.

The committee discussed the inadequacies of the total B12 assay as a gold standard test for deficiency. For example, low total B12 concentration does not necessarily indicate deficiency and high concentration does not necessarily exclude deficiency. In addition, it is not known how vitamin B12 levels in the peripheral blood correlate with levels in the central nervous system. The committee also highlighted the unmet need of people with borderline total B12 results, who do not receive a diagnosis of deficiency, but may require further testing. In the absence of evidence clearly supporting any single test or combination of tests over another, the committee considered the advantages and disadvantages of the different test options available.

Initial tests

In clinical practice, diagnostic blood testing is usually offered to a person suspected of having a vitamin B12 deficiency, because many of the signs and symptoms are non-specific, making it difficult to diagnose in the absence of other clinical evidence. However, the testing strategy used is not standard. The committee agreed that vitamin B12 deficiency should be suspected in anybody with at least one risk factor and at least one sign or symptom, because these people are more likely to have a vitamin B12 deficiency. However, the committee also considered that people often present with one sign or symptom in the absence of any risk factors, or risk factors may be unknown. The committee agreed that clinical judgement should therefore be used when deciding whether to test in these cases. See recommendations on when to suspect deficiency and the committee discussion of risk factors, signs and symptoms in evidence review B.

In current practice, when a test is ordered for suspected vitamin B12 deficiency, most laboratories measure total B12 as the initial test because it is well established and readily available compared to other testing methods. The committee considered that the active B12 test is a similar measure to total B12 as it is another direct marker of vitamin B12 status. Therefore, it would be more appropriate as an alternative first line test than as a second line test, whereas metabolic markers such as MMA or homocysteine would be more appropriate second line tests.

The committee discussed whether active B12 should be recommended over total B12 as a first line test. While they agreed that in their experience active B12 may be more accurate because it measures the active form of B12 which is used by the body, whereas total B12 also measures the inactive form, they noted that it is also significantly more expensive. Changing from the total B12 to active B12 would be a significant change in current practice and there was insufficient evidence to support any benefit of active B12 over total B12 or vice versa. Therefore, the committee decided to recommend use of either total B12 or active B12 as the first line test for suspected vitamin B12 deficiency. However, the committee acknowledged that active B12 is a more reliable test during pregnancy and breastfeeding, when total B12 concentrations in the body fall even when there is no deficiency, so they recommended active B12 as the only test for this group.

For people suspected of a vitamin B12 deficiency because of recreational nitrous oxide use, no evidence was identified, and the committee agreed by informal consensus based on their experience and expertise that plasma homocysteine is the only test that should be offered. This is because nitrous oxide inactivates vitamin B12, leading to dissociation from

methionine synthase and inactivation of apo-Methionine synthase. Inactivation of the enzyme leads to the impairment of homocysteine remethylation to methionine, creating a build-up of homocysteine. Highly elevated homocysteine is seen first in people who abuse nitrous oxide, followed by a slow decrease in total and active B12, as well as slight elevations in MMA. Vitamin B12 concentrations as measured by the total or active B12 test in people who use recreational nitrous oxide may therefore remain within the reference range. The committee also noted that homocysteine testing involves special tubes for sampling and not all primary care practices would have these available. In these circumstances the person should be referred to hospital for testing.

The committee acknowledged the limitations of the evidence identified on diagnostic accuracy and the lack of evidence identified on the effectiveness of different testing strategies. The committee agreed that there is a need for evidence on the long-term outcomes of the different testing strategies including total B12, active B12, methylmalonic acid and homocysteine and therefore made a research recommendation.

Interpreting test results

The committee discussed possible circumstances in which interpretation of total or active B12 tests may differ. One example is when a person has been taking vitamin B12 supplements prior to the sample being taken. This is because, depending on the amount of B12 contained in the supplement, they can raise vitamin B12 concentration in the blood and potentially mask a deficiency.

Women using contraceptives pills have significantly lower total and active B12 concentrations. The mechanism for the observed decrease is not clear and this may not reflect a functional deficiency. For interpretation of total or active B12 tests in this group, clinical judgement would be required, and further MMA testing may appropriate.

The committee considered how the test manufacturer reference ranges for the diagnostic tests were derived and what the implications may be for different patient groups. The total and active B12 tests provided by different manufacturers have not been standardised or harmonised, and reference intervals, when applied uniformly to different patient cohorts, have the potential to lead to misdiagnosis.

Women and people who are pregnant and breastfeeding are usually excluded from reference populations; therefore, manufacturer reference ranges may not be appropriate for use in these groups. The committee noted that as pregnancy progresses, the level of total B12 decreases, but may not necessarily reflect a deficient state. The committee considered that for this reason, test results should be interpreted with caution during pregnancy or breastfeeding and further testing may be required.

The committee noted that populations upon which the reference ranges are based may not represent the ethnic diversity of patients. The committee were aware of evidence from large UK cohort studies showing that vitamin B12 concentrations are higher in people from a Black family background[17, 21] Therefore, if reference ranges derived predominantly in white people are applied to this group, deficiency could be missed. The committee considered that for this reason, test results should be interpreted with caution in people from a Black family background.

The committee agreed that the general lack of evidence regarding the utility of the test reference ranges in ethnically diverse populations, different age groups, pregnancy and breastfeeding creates challenges in interpreting test results and diagnosing vitamin B12 deficiency. The committee hoped that by recommending further research in this area, future

studies will establish the most appropriate reference ranges for use in different patient groups.

The committee considered that macro B12 gives elevated serum B12 concentrations and in some cases this elevation masks a deficiency. The phenomenon is caused by immune complexes between serum immunoglobulins and B12 vitamin binding protein (macro-B12), where B12 bound to the immunoglobulins isn't metabolically available. In these cases, further tests with active B12 or MMA could be used, but high results should not be ignored if the person is not taking a B12 supplement as they can indicate underlying liver disease or haematologic malignancy. The committee discussed whether a specific recommendation on the presence of macro B12 would be of any value to users of this guideline. The committee agreed that it is the laboratory's responsibility to produce technically valid results and the clinician's responsibility to query with the laboratory any results that don't match the clinical picture. Analytical interference in immunoassays is common and not specific to B12 assays. Therefore, the committee decided not to make a recommendation.

Thresholds for test results

The evidence did not clearly support any particular cut-offs for total B12 or active B12 tests. The committee noted the difficulties in setting cut-offs, that test manufacturer cut-offs and chemistry platforms are not standardised. If the manufacturer cut-offs were to be recommended, it would have to be accepted that they are not interchangeable. In addition, units of measurement for total B12 are not standardised, with some laboratories using ng/L and others using pmol/L. Therefore, the committee agreed that for clarity, recommended cut offs for total B12 should be made for both units of measurement.

The committee discussed the option of setting high cut-offs to maximise sensitivity and reduce the number of second line tests required. The committee considered that high cut-offs would increase the risk of false positive results. However, people would usually be symptomatic in order to be tested and treatment is neither harmful nor expensive. The committee also noted that in current practice, people with test results close to the cut-off values would usually be offered treatment. Therefore, the committee decided to recommend relatively high cut-offs of 180 ng/L, or 133 pmol/L for total B12 and 25 pmol/L for active B12 for test positivity. If a homocysteine test is used, the committee agreed that clinical judgement should be used to determine which reference range to use, as these vary between different test manufacturers.

The committee also defined a range of values in which the results of the first line tests are not an adequate indicator of deficiency on their own, but when combined with further evidence in the form of a second line test, indicate a deficiency. The committee deliberated over the upper threshold of this 'grey zone', weighing the risk of false negative results against the impact of offering more people additional testing. The committee considered that the more second line tests are requested, the longer the turn-around time for results, which may impact negatively on patients. They also considered the cost impact of offering more second line tests. On the other hand, many people whose results currently fall into the grey zone, go on to have more appointments, referrals and diagnostic tests before receiving a diagnosis of B12 deficiency. In these cases, a higher threshold for second line testing could reduce the treatment delay for the person under investigation, as well as being cost saving. The range of test results on which a second line test could be offered was defined as 180 to 350 ng/L or 133 to 258 pmol/L for total B12 and 25 to 70 pmol/L for active B12, or 35 to 70 pmol/L for active B12 during pregnancy and breastfeeding.

The committee agreed that for people with results above the upper threshold, vitamin B12 deficiency is unlikely. The committee made a recommendation to explain this to the person

under investigation. The committee considered the exception that in a small number of people, a high vitamin B12 concentration caused by increased holohaptocorrin could mask a deficient state. However, they agreed that this is a rare occurrence and would not be relevant to the vast majority of people.

Given the lack of high-quality evidence for the diagnostic accuracy of the total B12 and active B12 tests and the issues identified by the committee on their reliability, the committee considered the risk of false negative results and how these should be identified and managed. The committee agreed that if signs and symptoms persist 3 to 6 months later, despite a negative test and no alternative diagnosis has been identified, it is possible that the result may have been a false negative. Therefore, the committee recommended consideration of repeat testing or testing MMA.

The committee considered the potential impact on the person of repeating the testing process and the possibility of creating health anxiety. The committee agreed that careful discussion with the person is needed before tests are repeated.

As reference ranges are generally higher in people from a Black family background, this group may need treatment even if blood test results show they are not deficient. The committee recommended that this is taken into account when interpreting test results together with symptoms, signs and risk factors and treatment should be considered in this group if their test result is indeterminate.

Actions or second line tests

The committee discussed homocysteine and MMA as possible second line tests to be carried out when first line test results are borderline/inconclusive. Several practical issues with the homocysteine test were identified, including the requirement for stabilising tubes if samples cannot be transported immediately to the laboratory on ice, which not all hospitals have, and the requirement of a second blood sample. Results of the homocysteine test are more difficult to interpret in people with folate, B6 or B2 deficiency and in people with renal impairment because these conditions also affect homocysteine levels. In addition, the committee considered the additional cost of the homocysteine test (see section below). MMA does not require stabilising tubes and can be performed on the original blood sample taken for the first line test. Avoiding the need for a second blood sample would speed up the diagnostic process and would involve less burden on the person being tested.

Therefore, the committee decided to recommend that MMA is considered as a second line test if first line test results fall within the ‘grey zone’ for diagnosis (180-350 ng/L for total B12; 25-70 pmol/L for active B12; 35-70 pmol/L for active B12 during pregnancy and breastfeeding) for people who have signs or symptoms that suggest they have deficiency.

The committee considered circumstances in which treatment should be considered with or without a further test with MMA, and that if the decision is to do the test, then starting treatment without waiting for the test results should be considered. These included people with conditions that may deteriorate rapidly and have a major effect on quality of life, such as neurological or haematological conditions like ataxia or anaemia; older adults with cognitive impairment; those likely to have irreversible vitamin B12 deficiency, such as autoimmune gastritis or those with positive anti-intrinsic factor antibodies; those likely to develop irreversible deficiency due to an operation such as gastrectomy, ileal terminal resection or bariatric surgery; and those who are pregnant or breastfeeding. This is because a delay in treatment could adversely affect quality of life or in the case of pregnancy and breastfeeding, the baby’s quality of life.

The committee also discussed the common scenario of 'incidental findings', when a person who has no signs or symptoms of vitamin B12 deficiency and is not suspected of having a deficiency but has their B12 levels measured as part of a routine blood investigation, such as a preoperative assessment or general health check. The committee agreed that for those with indeterminate test results without signs or symptoms, the initial test should be repeated in 6 months or sooner if signs or symptoms of vitamin B12 deficiency develop. The committee stressed the importance of people returning to their healthcare professionals if they develop symptoms of deficiency before the repeat test at 6 months.

The committee considered that, other than for diagnosing nitrous oxide-induced vitamin B12 deficiency, homocysteine may be useful in difficult to diagnose cases and in specialist centres, later on in the diagnostic pathway, but did not recommend its use within the context of the testing process for this guideline.

1.3.4 Cost effectiveness and resource use

Published economic evidence – first-line testing

One economic evaluation was included for this review which compared the cost effectiveness of five different strategies for diagnosing and treating B12 deficiency in adult patients hypothetically presenting with new unexplained fatigue in a primary care setting:

- Total B12 test – treat with oral B12
- Total B12 test – treat with parenteral B12
- No test – treat with oral B12
- No test – treat with parenteral B12
- No test – no treatment.

However, it was assessed as partially applicable with potentially serious quality limitations.

This study was based in Australia. For the utility data, the UK tariff was not used for EQ-5D; the utility scores were derived using hypothetical state scenarios which may not be comparable to one obtained by using patient data however they were based on clinical expert opinion. Another concern was that the primary symptom that was considered was fatigue, however this is only one non-specific symptom which may be related to B12 deficiency, so this study did not capture all potential B12 deficient patients.

It was classed as having potentially serious limitations. Firstly, the cost of misdiagnosis such as repeat GP visits was not included, which could have a significant impact of the results. There was also uncertainty regarding the baseline prevalence of B12 deficiency and the utility data were based on expert opinion. Another factor was the short time horizon of three months. In some cases, B12 deficiency needs longer term treatment and in the case of autoimmune gastritis (also known as pernicious anaemia) this would be potentially lifelong.

In terms of the costs, GP consultation fee, total B12 test, specimen fees, medicine costs and administration costs were included in the model. The most cost-effective strategies in the study were 'do not test, treat with oral B12', but the committee were concerned about misdiagnosis of other conditions in this broad population.

Original cost-effectiveness analysis – second-line testing

The committee members had stated due to the poor performance of total B12 and active B12 as first line tests it would be important to explore the cost effectiveness of using a second line test. This would be applicable for people whose test result is in a predefined indeterminate range. The second line test that would be used is methylmalonic acid testing (MMA) which costs more than both total B12 and active B12 however elevated concentrations of MMA are

considered the most representative marker of metabolic vitamin B12 hence why this test was preferred as a second-line test. Due to the high economic and clinical importance of this question, an original economic analysis was undertaken to determine whether the use of 'MMA testing, treat positive' was cost effective vs 'no MMA testing, no treatment' for people who have had an initial first-line test result (either active or total B12) within the indeterminate region.

In the absence of empirical data, the utility gain from B12 replacement therapy based on expert opinion from the published economic evaluation was used. MMA was assumed to be 100% sensitive and specific in the absence of an accepted reference standard.

The lowest cost intervention was the 'no MMA testing, no treatment' strategy. The strategy with the greatest benefits in terms of QALYs was 'MMA testing, treat positive'.

In the base case, the incremental cost per QALY for testing with MMA vs 'No testing, no treatment' was £3,946. Varying the prevalence, MMA cost and the time horizon of treatments was explored to check how likely it is that MMA testing would be cost effective. In all instances the cost per QALY for MMA vs 'No test, no treatment' was less than £20,000.

As part of the sensitivity analysis, the strategy of 'no MMA testing, treat all' was investigated. For this analysis there was assumed to be no difference in health outcomes as all B12 deficient patients are given the appropriate treatment in the 'MMA testing' and 'no MMA testing, treat all' strategies. For people who were incorrectly given B12 treatment there is no utility loss as it is assumed B12 treatment is not toxic and unlikely to cause any worsening symptoms. For people who do not have underlying B12 deficiency there is a simplifying assumption that their symptoms are transient and self-limiting and therefore their utility improves gradually over three months (identical to the treated utility score of B12 deficient patients).

Assuming the base case parameters, 'no MMA testing, treat all' appears to be the lowest cost option. However, there was uncertainty in the results which are available in Appendix I. Furthermore, if the initial licensed dose of Orobalin was used (4000mcg daily), testing MMA would become more preferable due to the increased costs of treatment. The committee expressed that this strategy was not ideal because patients would be left with an uncertain diagnosis. Also, there was a risk that for some people, treatment could be continued long-term unnecessarily. In all scenarios where treatment continued for one year, MMA testing was the lowest cost option.

Limitations of the original cost-effectiveness analysis

The diagnostic and treatment pathway are uncertain in current practice. MMA is not commonly conducted. It is thought that in some locations this population might be treated with B12 without further testing whereas in other locations they would not be considered vitamin B12 deficient at all. The committee suspect the latter is more predominant.

Limited evidence was identified to inform the model. There was only one published paper that was used to inform the prevalence of elevated MMA in the population that have indeterminate results. A further assumption that was made was that MMA will be 100% accurate (in the absence of a better reference standard). In instances where the test may not be 100% accurate, there is a potential cost implication by treating false positive cases of deficiency, and the risk that an alternative diagnosis has been missed. The treatments for B12 deficiency are not thought to be toxic and they are well tolerated.

In addition to this, the model only comprised of MMA testing; there was no further differential diagnostic testing to determine the underlying cause of the B12, such as autoimmune gastritis, which would influence treatment choice. In the absence of macrocytic anaemias or pernicious anaemia the use of parenteral hydroxocobalamin treatment is considered off label. Also, for diet related B12 deficiency, parenteral treatment is considered off-label.

Therefore, parenteral hydroxocobalamin treatment was not included within the model despite in some circumstances it being appropriate.

For the licensing of Orobalin, the initial dose is 4000mcg daily until remission. However, there is uncertainty about how long the time taken to remission is and how to assess remission. The experience of the committee is that for newly diagnosed people with B12 deficiency, when cyanocobalamin 1000mcg tablets are prescribed the starting dose is one tablet a day rather than four tablets a day. This was the treatment dose within the model for people who have B12 deficiency. This higher dose 1000mcg cyanocobalamin was less costly than the 50mcg cyanocobalamin tablet and considered to be more suitable for treatment despite the BNF (British National Formulary) dose for B12 deficiency being 50mcg to 150mcg cyanocobalamin. The committee agreed that the 1000mcg dose would be preferred over the use of a 50mcg-150mcg dose.

Within the model, there is a simplifying assumption that for the patients who do not have underlying B12 deficiency, their symptoms are transient and self-limiting and therefore their utility improves gradually over three months (which is the same as the treated utility score). For people incorrectly given B12 treatment, there is no utility loss as it is assumed B12 treatment is not toxic and unlikely to cause any worsening symptoms. For the B12 deficient population within this group there will be no utility/quality of life improvement.

This model assumes that for truly B12 deficient people in the ‘no testing, no treatment’ strategy will consult at least once more with their GP surgery within the three months of the indeterminate active or total B12 test result. There is an assumption that this appointment will lead to the appropriate diagnosis of B12 deficiency. The committee members believe that one GP appointment is very conservative due to the variability of symptoms whereas from the collective experiences of the committee there may be repeated GP visits or secondary care referrals. If costs of complications and hospitalisations due to B12 deficiency were included, it would favour MMA testing as the preferred cost-effective strategy due to MMA testing confirming a B12 deficiency diagnosis and hence treatment beginning. The committee agree that MMA testing if indicated would be preferable over a secondary care referral due to investigation delays and the additional cost of the referral.

It is unlikely that the utility improvement based on expert opinion would reflect the wide variation in quality of life experienced by B12-deficient patients. However, the committee thought it would be appropriate for modelling as it may be reflective of a large proportion of B12-deficient patient and reasonable to use. The committee also believed that people with autoimmune gastritis may have higher utility gains with appropriate diagnosis and treatment therefore testing with MMA will be more cost effective.

Recommendations

The committee decided to recommend either total B12 or active B12 as the initial test for suspected vitamin B12 deficiency. Prices obtained from committee members indicated that active B12 testing (£18) was significantly more expensive than total b12 testing (£2). The committee members expressed the view that active B12 provides a better indicator of B12 deficiency than total B12, however the cost effectiveness of these tests is uncertain. These two tests are less costly than other tests, and they are used routinely. There was not enough evidence to recommend a change in practice to recommend active B12 as the first line test.

In terms of the de-novo model, the committee agreed that the original model is simplified and cannot capture all the relevant scenarios due to the lack of clinical data available. However, they agreed that there is enough evidence to support a ‘consider’ recommendation for the use of MMA testing for people who have an indeterminate first line test result. This will support timely diagnosis and treatment.

There was consensus amongst the committee that MMA testing should not be offered to everyone that presents with an indeterminate result. The committee prefer clinicians utilising

their clinical judgement for individual management. For example, incidental findings in people identified by screening tests who may not have any symptoms may not warrant an MMA test as dietary advice may be sufficient for this population. For people who have an obvious cause of deficiency such as drug related adverse effect such as metformin then it may be appropriate to treat without further investigations. For people that may have more severe symptoms and potentially more risk factors for B12 deficiency it is thought that this would increase the likelihood of MMA testing being requested.

MMA testing was recommended as a 'consider' second-line test due to the limitations of the clinical evidence as well as the potential resource impact in the absence of more robust data. It was also considered appropriate to start treatment as an alternative to testing in some patients, which is reasonable as there was uncertainty in the model about whether second line testing or treatment was the more cost effective.

Resource impact

In terms of the initial diagnostic B12, the recommendations will not change practice, which is usually total or active B12.

Testing for MMA will increase due to the greater awareness of this test and the recommendation itself. By limiting the use of MMA as a confirmatory test for people who have an indeterminate first line test result, this will limit resource impact. MMA testing will aid timely diagnosis and treatment whilst also potentially saving resources by reducing the number of referrals to secondary care and reduce inappropriate investigations.

1.3.5 Other factors the committee took into account

Timing of diagnostic tests

The committee discussed the implications of the timing of diagnostic tests for people with suspected vitamin B12 deficiency. If blood samples are taken after a person has started treatment, then the test results are unlikely to accurately reflect the person's true vitamin B12 status. Therefore, the committee recommended that blood samples for testing should be taken before treatment is started.

The committee were aware that people may use vitamin B12 supplements, which can contain doses of vitamin B12 between 100 and 2000mcg. The committee considered that some higher doses could affect or invalidate test results as they can raise levels of vitamin B12 in the body, potentially disguising a deficiency. The committee discussed whether it would be feasible to delay testing until the effects of the supplement are eliminated. However, the committee concluded that there are too many variables such as different strengths, durations, preparations and impaired absorption, to be able to accurately determine the correct washout period. It was therefore recommended that use of supplements should be investigated and taken into consideration when interpreting the results of the test.

It was noted that serum B12 tests are usually processed relatively quickly and often on the same day when carried out in hospital, although MMA tests take longer. Delays in testing occur more commonly in primary care, as a result of delays in obtaining the blood sample rather than processing the results.

The committee also considered people who are severely ill and who may require urgent treatment. The committee agreed that those with severe symptoms, for example those with severe megaloblastic anaemia or subacute degeneration of the spinal cord should not wait for the results of diagnostic tests before starting treatment and made a recommendation to emphasise this. The committee considered that the recommendation to take blood samples for testing vitamin B12 deficiency before starting treatment would not prevent treatment

before the test results are processed in these cases, but would ensure that the blood samples taken do not lead to inaccurate results.

1.3.6 Recommendations supported by this evidence review

This evidence review supports recommendations 1.3.1 to 1.3.15 and the research recommendation on what are the long-term outcomes for people with suspected vitamin B12 deficiency when comparing testing of total serum B12 (serum cobalamin), active B12 (holotranscobalamin), methylmalonic acid (MMA) or homocysteine.

1.4 References

1. Bolann BJ, Solli JD, Schneede J, Grottum KA, Loraas A, Stokkeland M et al. Evaluation of indicators of cobalamin deficiency defined as cobalamin-induced reduction in increased serum methylmalonic acid. Clinical Chemistry. 2000; 46(11):1744-1750

2. Bondu JD, Nellickal AJ, Jeyaseelan L, Geethanjali FS. Assessing diagnostic accuracy of serum holotranscobalamin (active-B12) in comparison with other markers of vitamin B12 deficiency. Indian Journal of Clinical Biochemistry. 2020; 35(3):367-372

3. Campos AJ, Risch L, Nydegger U, Wiesner J, Dyck MVV, Seger C et al. Diagnostic characteristics of 3-parameter and 2-parameter equations for the calculation of a combined indicator of vitamin B12 status to predict cobalamin deficiency in a large mixed patient population. Clinical Laboratory. 2020; 66(10):01

4. Goringe A, Ellis R, McDowell I, Vidal-Alaball J, Jenkins C, Butler C et al. The limited value of methylmalonic acid, homocysteine and holotranscobalamin in the diagnosis of early B12 deficiency. Haematologica. 2006; 91(2):231-234

5. Heil SG, de Jonge R, de Rotte MC, van Wijnen M, Heiner-Fokkema RM, Kobold AC et al. Screening for metabolic vitamin B12 deficiency by holotranscobalamin in patients suspected of vitamin B12 deficiency: a multicentre study. Annals of Clinical Biochemistry. 2012; 49(pt2):184-189

6. Herrmann W, Obeid R. Utility and limitations of biochemical markers of vitamin B12 deficiency. European Journal of Clinical Investigation. 2013; 43(3):231-237

7. Holleland G, Schneede J, Ueland PM, Lund PK, Refsum H, Sandberg S. Cobalamin deficiency in general practice. Assessment of the diagnostic utility and cost-benefit analysis of methylmalonic acid determination in relation to current diagnostic strategies. Clinical Chemistry. 1999; 45(2):189-198

8. Jarquin Campos A, Risch L, Nydegger U, Wiesner J, Vazquez Van Dyck M, Renz H et al. Diagnostic Accuracy of holotranscobalamin, vitamin B12, methylmalonic acid, and homocysteine in detecting B12 deficiency in a large, mixed patient population. Disease Markers. 2020; 2020:7468506

9. Jones K, Burns A. Unit costs of health and social care 2021. Canterbury. Personal Social Services Research Unit University of Kent, 2021. Available from: https://www.pssru.ac.uk/project-pages/unit-costs/unit-costs-of-health-and-social-care-2021/

10. Matchar DB, Feussner JR, Millington DS, Wilkinson RH, Jr., Watson DJ, Gale D. Isotope-dilution assay for urinary methylmalonic acid in the diagnosis of vitamin B12 deficiency. A prospective clinical evaluation. Annals of Internal Medicine. 1987; 106(5):707-710

11. Matchar DB, McCrory DC, Millington DS, Feussner JR. Performance of the serum cobalamin assay for diagnosis of cobalamin deficiency. American Journal of the Medical Sciences. 1994; 308(5):276-283

12. Mnatzaganian G, Karnon J, Moss JR, Elshaug AG, Metz M, Frank OR et al. Informing disinvestment with limited evidence: cobalamin deficiency in the fatigued. International Journal of Technology Assessment in Health Care. 2015; 31(3):188-196

13. Moelby L, Rasmussen K, Jensen MK, Pedersen KO. The relationship between clinically confirmed cobalamin deficiency and serum methylmalonic acid. Journal of Internal Medicine. 1990; 228(4):373-378

14. National Institute for Health and Care Excellence. Active B12 assay for diagnosing vitamin B12 deficiency. London. National Institute for Health and Care Excellence, 2015. Available from: https://www.nice.org.uk/advice/mib40

15. National Institute for Health and Care Excellence. Developing NICE guidelines: the manual [updated January 2022]. London. National Institute for Health and Care Excellence, 2014. Available from: http://www.nice.org.uk/article/PMG20/chapter/1%20Introduction%20and%20overview

16. NHS Business Services Authority. NHS electronic drug tariff December 2022. 2022. Available from: https://www.nhsbsa.nhs.uk/pharmacies-gp-practices-and-appliance-contractors/drug-tariff Last accessed: 09/12/2022.

17. O'Logbon J, Crook M, Steed D, Harrington DJ, Sobczynska-Malefora A. Ethnicity influences total serum vitamin B(12) concentration: a study of Black, Asian and White patients in a primary care setting. Journal of Clinical Pathology. 2022; 75(9):598-604

18. Organisation for Economic Co-operation and Development (OECD). Purchasing power parities (PPP). 2021. Available from: https://data.oecd.org/conversion/purchasing-power-parities-ppp.htm Last accessed: 01/06/2022.

19. Schrempf W, Eulitz M, Neumeister V, Siegert G, Koch R, Reichmann H et al. Utility of measuring vitamin B12 and its active fraction, holotranscobalamin, in neurological vitamin B12 deficiency syndromes. Journal of Neurology. 2011; 258(3):393-401

20. Sobczynska-Malefora A, Gorska R, Pelisser M, Ruwona P, Witchlow B, Harrington DJ. An audit of holotranscobalamin ("Active" B12) and methylmalonic acid assays for the assessment of vitamin B12 status: application in a mixed patient population. Clinical Biochemistry. 2014; 47(12):82-86

21. Sobczynska-Malefora A, Katayev A, Steed D, O'Logbon J, Crook M, Harrington DJ. Age- and ethnicity-related reference intervals for serum vitamin B(12). Clinical Biochemistry. 2023; 111:66-71

Appendices

Appendix A – Review protocols

A.1 Diagnostic accuracy

Review protocol for accuracy of diagnostic tests

ID	Field	Content
0.	PROSPERO registration number	CRD42022321545
1.	Review title	What is the diagnostic accuracy of tests (including the serum cobalamin assay and holotranscobalamin, methylmalonic acid and homocysteine tests) for diagnosing vitamin B12 deficiency?
2.	Review question	What is the diagnostic accuracy of tests (including the serum cobalamin assay and holotranscobalamin, methylmalonic acid and homocysteine tests) for diagnosing vitamin B12 deficiency?
3.	Objective	To determine the diagnostic accuracy of tests for diagnosing vitamin B12 deficiency.
4.	Searches	The following databases (from inception) will be searched: Cochrane Central Register of Controlled Trials (CENTRAL) Cochrane Database of Systematic Reviews (CDSR) Embase MEDLINE English language studies Human studies

		Other searches: Inclusion lists of systematic reviews The searches may be re-run 6 weeks before the final committee meeting and further studies retrieved for inclusion if relevant. The full search strategies will be published in the final review. Medline search strategy to be quality assured using the PRESS evidence-based checklist (see methods chapter for full details).
5.	Condition or domain being studied	Vitamin B12 deficiency
6.	Population	Inclusion: Adults with suspected vitamin B12 deficiency Exclusion: people taking vitamin B12 supplements Stratify by: • Age (adults 16/18 years and older; older adults 65 years and older) • Third trimester of pregnancy (third trimester; first two trimesters and not pregnant) • Ethnicity (Afro-Caribbean; other) • Sex (male; female) (study defined) for Homocysteine test only
7.	Test	The following as stand-alone tests, in combination or as staged tests: Serum cobalamin assay Holotranscobalamin test Methylmalonic acid test (including urinary) Homocysteine test

		Strata: reference ranges as defined by the studies
8.	Reference standard	Reference standards defined by the studies
9.	Types of study to be included	Inclusion: Cross-sectional studies Diagnostic accuracy observational cohort studies Systematic reviews of the above Exclusion: Case-control studies
10.	Other exclusion criteria	Studies that do not report sensitivity and specificity, or insufficient data to derive these values Non-English language studies. Conference abstracts.
11.	Context	NA
12.	Primary outcomes (critical outcomes)	Sensitivity 90% for first line and 80% for second line tests Specificity 70% for first line and 90% for second line tests Raw data to calculate 2x2 tables to calculate sensitivity and specificity (number of true positives, true negatives, false positives and false negatives). Predictive values Likelihood ratios
13.	Data extraction (selection and coding)	All references identified by the searches and from other sources will be uploaded into EPPI reviewer and de-duplicated. 10% of the abstracts will be reviewed by two reviewers, with any disagreements resolved by discussion or, if necessary, a third independent reviewer.

		The full text of potentially eligible studies will be retrieved and will be assessed in line with the criteria outlined above. A standardised form will be used to extract data from studies (see Developing NICE guidelines: the manual section 6.4). 10% of all evidence reviews are quality assured by a senior research fellow. This includes checking: papers were included /excluded appropriately a sample of the data extractions correct methods are used to synthesise data a sample of the risk of bias assessments Disagreements between the review authors over the risk of bias in particular studies will be resolved by discussion, with involvement of a third review author where necessary. Study investigators may be contacted for missing data where time and resources allow.	
14.	Risk of bias (quality) assessment	Risk of bias quality assessment will be assessed using QUADAS-2. Assessment will be independently quality assured by a second reviewer. Disagreements between the reviewers will be resolved by discussion, with involvement of a third party where necessary.	
15.	Strategy for data synthesis	Where possible data will be meta-analysed where appropriate (if at least 3 studies reporting data at the same diagnostic threshold) in WinBUGS. Summary diagnostic outcomes will be reported from the meta-analyses with their 95% confidence intervals in adapted GRADE tables. Heterogeneity will be assessed by visual inspection of the sensitivity and specificity plots and summary area under the curve (AUC) plots. If meta-analysis is not possible, data will be presented as individual values in adapted GRADE profile tables and plots of un-pooled sensitivity and specificity from RevMan software.	
16.	Analysis of sub-groups	Subgroups that will be investigated if heterogeneity is present: none	
17.	Type and method of review	☐	Intervention
		☒	Diagnostic
		☐	Prognostic

		☐	Qualitative
		☐	Epidemiologic
		☐	Service Delivery
		☐	Other (please specify)
18.	Language	English	
19.	Country	England	
20.	Anticipated or actual start date	28/03/2022	
21.	Anticipated completion date	01/11/2023	
22.	Stage of review at time of this submission	Review stage	Started / Completed
		Preliminary searches	☐ / ☐
		Piloting of the study selection process	☐ / ☐
		Formal screening of search results against eligibility criteria	☐ / ☐
		Data extraction	☐ / ☐
		Risk of bias (quality) assessment	☐ / ☐
		Data analysis	☐ / ☐
23.	Named contact	5a. Named contact	

		National Guideline Centre 5b Named contact e-mail PerniciousAnaemia@nice.nhs.uk 5e Organisational affiliation of the review National Institute for Health and Care Excellence (NICE) and National Guideline Centre
24.	Review team members	From the National Guideline Centre: Carlos Sharpin [Guideline lead] Maria Smyth [Senior systematic reviewer] Toby Sands [Systematic reviewer] Aamer Jawed [Health economist] Stephen Deed [Information specialist] Katie Tuddenham [Project manager]
25.	Funding sources/sponsor	This systematic review is being completed by the National Guideline Centre which receives funding from NICE.
26.	Conflicts of interest	All guideline committee members and anyone who has direct input into NICE guidelines (including the evidence review team and expert witnesses) must declare any potential conflicts of interest in line with NICE's code of practice for declaring and dealing with conflicts of interest. Any relevant interests, or changes to interests, will also be declared publicly at the start of each guideline committee meeting. Before each meeting, any potential conflicts of interest will be considered by the guideline committee Chair and a senior member of the development team. Any decisions to exclude a person from all or part of a meeting will be documented. Any changes to a member's declaration of interests will be recorded in the minutes of the meeting. Declarations of interests will be published with the final guideline.
27.	Collaborators	Development of this systematic review will be overseen by an advisory committee who will use the review to inform the development of evidence-based recommendations in line with section 3 of Developing NICE

		guidelines: the manual. Members of the guideline committee are available on the NICE website: Project documents | Vitamin B12 deficiency, including pernicious anaemia: diagnosis and management | Guidance | NICE	
28.	Other registration details		
29.	Reference/URL for published protocol	https://www.crd.york.ac.uk/prospero/display_record.php?	
30.	Dissemination plans	NICE may use a range of different methods to raise awareness of the guideline. These include standard approaches such as: notifying registered stakeholders of publication publicising the guideline through NICE's newsletter and alerts issuing a press release or briefing as appropriate, posting news articles on the NICE website, using social media channels, and publicising the guideline within NICE.	
31.	Keywords		
32.	Details of existing review of same topic by same authors		
33.	Current review status	☐	Ongoing
		☐	Completed but not published
		☐	Completed and published
		☐	Completed, published and being updated
		☐	Discontinued
34.	Additional information		
35.	Details of final publication	www.nice.org.uk	

A.21 Intervention

2 **Review protocol for diagnosis of vitamin B12 deficiency**

ID	Field	Content
0.	PROSPERO registration number	CRD42022308431
1.	Review title	What are the most clinically and cost-effective ways to diagnose vitamin B12 deficiency, including the serum cobalamin assay and holotranscobalamin, methylmalonic acid and homocysteine tests?
2.	Review question	What are the most clinically and cost-effective ways to diagnose vitamin B12 deficiency, including the serum cobalamin assay and holotranscobalamin, methylmalonic acid and homocysteine tests?
3.	Objective	To evaluate the most clinically and cost-effective way to diagnose vitamin B12 deficiency.
4.	Searches	The following databases (from inception) will be searched: • Cochrane Central Register of Controlled Trials (CENTRAL) • Cochrane Database of Systematic Reviews (CDSR) • Embase • MEDLINE • Epistemonikos Searches will be restricted by: • English language studies • Human studies Other searches:

		• Inclusion lists of systematic reviews The searches may be re-run 6 weeks before the final committee meeting and further studies retrieved for inclusion if relevant. The full search strategies will be published in the final review. Medline search strategy to be quality assured using the PRESS evidence-based checklist (see methods chapter for full details).
5.	Condition or domain being studied	Vitamin B12 deficiency
6.	Population	Inclusion: Adults with suspected vitamin B12 deficiency. Exclusion: None Stratify by: • Age (adults 16/18 years and older; older adults 65 years and older) • Third trimester of pregnancy (third trimester; first two trimesters and not pregnant) • Ethnicity (Afro-Caribbean; other) • Sex (male; female) (study defined) for Homocysteine test only
7.	Intervention	The following as stand-alone tests or in combination: • Serum cobalamin assay • Holotranscobalamin test • Methylmalonic acid test (including urinary) • Homocysteine test

		Treatment as a result of a positive test: • Vitamin B12 replacement 1. Hydroxocobalamin 2. Cyanocobalamin 3. Cobalamin/B12 Strata: reference ranges as defined by the studies
8.	Comparator	• All tests and combinations of tests compared with each other • No test (treatment only)
9.	Types of study to be included	• Randomised controlled trials • Systematic reviews of RCTs
10.	Other exclusion criteria	Cohort studies Non-English language studies Conference abstracts
11.	Context	NA
12.	Primary outcomes (critical outcomes)	All outcomes are considered equally important for decision making and therefore have all been rated as critical: • quality of life (such as EQ5D, SF36) • patient-reported outcomes (PROM scores including some/all symptoms): 1. fatigue 2. sleep 3. peripheral neuropathy 4. cognition 5. psychiatric symptoms 6. pain • haematological values • complications and adverse events

		1. mortality 2. bleeds 3. self-harm 4. nerve damage 5. frailty/falls 6. severe cognitive effects 7. postural hypotension • patient concern around unexpected lab results (health anxiety score) • incorrect/delayed diagnosis • inappropriate additional tests • adherence to treatment • education/work absence Time point: any time point available
13.	Data extraction (selection and coding)	All references identified by the searches and from other sources will be uploaded into EPPI reviewer and de-duplicated. 10% of the abstracts will be reviewed by two reviewers, with any disagreements resolved by discussion or, if necessary, a third independent reviewer. The full text of potentially eligible studies will be retrieved and will be assessed in line with the criteria outlined above. A standardised form will be used to extract data from studies (see Developing NICE guidelines: the manual section 6.4). 10% of all evidence reviews are quality assured by a senior research fellow. This includes checking: • papers were included /excluded appropriately • a sample of the data extractions • correct methods are used to synthesise data • a sample of the risk of bias assessments

		Disagreements between the review authors over the risk of bias in particular studies will be resolved by discussion, with involvement of a third review author where necessary. Study investigators may be contacted for missing data where time and resources allow.
14.	Risk of bias (quality) assessment	Risk of bias will be assessed using the appropriate checklist as described in Developing NICE guidelines: the manual. For Intervention reviews the following checklist will be used according to study design being assessed: Randomised Controlled Trial: Cochrane RoB (2.0) Systematic reviews: Risk of Bias in Systematic Reviews (ROBIS)
15.	Strategy for data synthesis	Pairwise meta-analyses will be performed using Cochrane Review Manager (RevMan5). Fixed-effects (Mantel-Haenszel) techniques will be used to calculate risk ratios for the binary outcomes where possible. Continuous outcomes will be analysed using an inverse variance method for pooling weighted mean differences. Heterogeneity between the studies in effect measures will be assessed using the I^2 statistic and visually inspected. An I^2 value greater than 50% will be considered indicative of substantial heterogeneity. Sensitivity analyses will be conducted based on pre-specified subgroups using stratified meta-analysis to explore the heterogeneity in effect estimates. If this does not explain the heterogeneity, the results will be presented pooled using random-effects. GRADEpro will be used to assess the quality of evidence for each outcome, taking into account individual study quality and the meta-analysis results. The 4 main quality elements (risk of bias, indirectness, inconsistency and imprecision) will be appraised for each outcome. Publication bias will be considered with the guideline committee, and if suspected will be tested for when there are more than 5 studies for that outcome. The risk of bias across all available evidence was evaluated for each outcome using an adaptation of the 'Grading of Recommendations Assessment,

		Development and Evaluation (GRADE) toolbox' developed by the international GRADE working group http://www.gradeworkinggroup.org/ Where meta-analysis is not possible, data will be presented and quality assessed individually per outcome.		
16.	Analysis of sub-groups	Subgroups that will be investigated if heterogeneity is present: none		
17.	Type and method of review	☒	Intervention	
		☐	Diagnostic	
		☐	Prognostic	
		☐	Qualitative	
		☐	Epidemiologic	
		☐	Service Delivery	
		☐	Other (please specify)	
18.	Language	English		
19.	Country	England		
20.	Anticipated or actual start date	28/01/2022		
21.	Anticipated completion date	01/11/2023		
22.	Stage of review at time of this submission	Review stage	Started	Completed
		Preliminary searches	☑	☐
		Piloting of the study selection process	☐	☐

		Formal screening of search results against eligibility criteria	☐	☐
		Data extraction	☐	☐
		Risk of bias (quality) assessment	☐	☐
		Data analysis	☐	☐
23.	Named contact	5a. Named contact National Guideline Centre 5b Named contact e-mail PerniciousAnaemia@nice.nhs.uk 5e Organisational affiliation of the review National Institute for Health and Care Excellence (NICE) and National Guideline Centre		
24.	Funding sources/sponsor	This systematic review is being completed by the National Guideline Centre which receives funding from NICE.		
25.	Conflicts of interest	All guideline committee members and anyone who has direct input into NICE guidelines (including the evidence review team and expert witnesses) must declare any potential conflicts of interest in line with NICE's code of practice for declaring and dealing with conflicts of interest. Any relevant interests, or changes to interests, will also be declared publicly at the start of each guideline committee meeting. Before each meeting, any potential conflicts of interest will be considered by the guideline committee Chair and a senior member of the development team. Any decisions to exclude a person from all or part of a meeting will be documented. Any changes to a member's declaration of interests		

		will be recorded in the minutes of the meeting. Declarations of interests will be published with the final guideline.	
26.	Collaborators	Development of this systematic review will be overseen by an advisory committee who will use the review to inform the development of evidence-based recommendations in line with section 3 of Developing NICE guidelines: the manual. Members of the guideline committee are available on the NICE website: Project documents | Vitamin B12 deficiency, including pernicious anaemia: diagnosis and management | Guidance | NICE	
27.	Other registration details	NA	
28.	Reference/URL for published protocol	https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42022308431	
29.	Dissemination plans	NICE may use a range of different methods to raise awareness of the guideline. These include standard approaches such as: • notifying registered stakeholders of publication • publicising the guideline through NICE's newsletter and alerts • issuing a press release or briefing as appropriate, posting news articles on the NICE website, using social media channels, and publicising the guideline within NICE.	
30.	Keywords		
31.	Details of existing review of same topic by same authors	NA	
32.	Current review status	☒	Ongoing
		☐	Completed but not published
		☐	Completed and published
		☐	Completed, published and being updated
		☐	Discontinued

33.	Additional information	NA
34.	Details of final publication	www.nice.org.uk

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1 Health economic review protocol

Review question	All questions – health economic evidence
Objectives	To identify health economic studies relevant to any of the review questions.
Search criteria	• Populations, interventions and comparators must be as specified in the clinical review protocol above. • Studies must be of a relevant health economic study design (cost–utility analysis, cost-effectiveness analysis, cost–benefit analysis, cost–consequences analysis, comparative cost analysis). • Studies must not be a letter, editorial or commentary, or a review of health economic evaluations. (Recent reviews will be ordered although not reviewed. The bibliographies will be checked for relevant studies, which will then be ordered.) • Unpublished reports will not be considered unless submitted as part of a call for evidence. • Studies must be in English.
Search strategy	A health economic study search will be undertaken using population-specific terms and a health economic study filter – see appendix B below.
Review strategy	Studies not meeting any of the search criteria above will be excluded. Studies published before 2006, abstract-only studies and studies from non-OECD countries or the USA will also be excluded. Each remaining study will be assessed for applicability and methodological limitations using the NICE economic evaluation checklist which can be found in appendix H of Developing NICE guidelines: the manual (2014).[15] **Inclusion and exclusion criteria** • If a study is rated as both 'Directly applicable' and with 'Minor limitations' then it will be included in the guideline. A health economic evidence table will be completed and it will be included in the health economic evidence profile. • If a study is rated as either 'Not applicable' or with 'Very serious limitations' then it will usually be excluded from the guideline. If it is excluded then a health economic evidence table will not be completed and it will not be included in the health economic evidence profile. • If a study is rated as 'Partially applicable', with 'Potentially serious limitations' or both then there is discretion over whether it should be included. **Where there is discretion** The health economist will make a decision based on the relative applicability and quality of the available evidence for that question, in discussion with the guideline committee if required. The ultimate aim is to include health economic studies that are helpful for decision-making in the context of the guideline and the current NHS setting. If several studies are considered of sufficiently high applicability and methodological quality that they could all be included, then the health economist, in discussion with the committee if required, may decide to include only the most applicable studies and to selectively exclude the remaining studies. All studies excluded on the basis of applicability or methodological limitations will be listed with explanation in the excluded health economic studies appendix below. The health economist will be guided by the following hierarchies. *Setting:* • UK NHS (most applicable). • OECD countries with predominantly public health insurance systems (for example, France, Germany, Sweden).

	• OECD countries with predominantly private health insurance systems (for example, Switzerland). • Studies set in non-OECD countries or in the USA will be excluded before being assessed for applicability and methodological limitations. *Health economic study type:* • Cost–utility analysis (most applicable). • Other type of full economic evaluation (cost–benefit analysis, cost-effectiveness analysis, cost–consequences analysis). • Comparative cost analysis. • Non-comparative cost analyses including cost-of-illness studies will be excluded before being assessed for applicability and methodological limitations. *Year of analysis:* • The more recent the study, the more applicable it will be. • Studies published in 2006 or later but that depend on unit costs and resource data entirely or predominantly from before 2006 will be rated as 'Not applicable'. • Studies published before 2006 will be excluded before being assessed for applicability and methodological limitations. *Quality and relevance of effectiveness data used in the health economic analysis:* • The more closely the clinical effectiveness data used in the health economic analysis match with the outcomes of the studies included in the clinical review the more useful the analysis will be for decision-making in the guideline.

Appendix B Literature search strategies

The literature searches for these reviews are detailed below and complied with the methodology outlined in Developing NICE guidelines: the manual.[15]

For more information, please see the Methodology review published as part of the accompanying documents for this guideline.

B.1 What is the diagnostic accuracy of tests (including the serum cobalamin assay and holotranscobalamin, methylmalonic acid and homocysteine tests) for diagnosing vitamin B12 deficiency?

B.1.1 Clinical search literature search strategy

Searches were constructed using a PICO framework where population (P) terms were combined with Intervention (I) and in some cases Comparison (C) terms. Outcomes (O) are rarely used in search strategies as these concepts may not be indexed or described in the title or abstract and are therefore difficult to retrieve.

Table 17: Database parameters, filters and limits applied

Database	Dates searched	Search filter used
Medline (OVID)	1946 – 16 December 2022	Exclusions (animal studies, letters, comments, editorials, case studies/reports, randomised controlled trials) English language

Database	Dates searched	Search filter used
Embase (OVID)	1974 – 16 December 2022	Exclusions (animal studies, letters, comments, editorials, case studies/reports, conference abstracts, randomised controlled trials) English language
The Cochrane Library (Wiley)	Cochrane Database of Systematic Reviews to Issue 12 of 12, 16 December 2022 Cochrane Central Register of Controlled Trials to Issue 12 of 12, 16 December 2022	Exclusions (clinical trials, conference abstracts)
Epistemonikos (The Epistemonikos Foundation)	Inception to 16 December 2022	Systematic review Exclusions (Cochrane reviews)

1 **Medline (Ovid) search terms**

1.	exp Vitamin B 12 Deficiency/
2.	((b12 or b 12 or cobalamin* or c?anocobalamin* or transcobalamin*) adj4 (deficien* or malabsor* or absor* or lack* or diminish* or low* or level* or abnormal* or deficit or disorder* or inadequa* or hypovitaminosis or hypo vitaminosis or avitaminosis)).ti,ab.
3.	exp Macrocytic Anemia/
4.	((b12 or b 12 or macrocytic or megaloblastic or pernicious or addison*) adj3 (anemia* or anaemia*)).ti,ab.
5.	Intrinsic Factor/
6.	intrinsic factor.ti,ab.
7.	or/1-6
8.	letter/
9.	editorial/
10.	news/
11.	exp historical article/
12.	Anecdotes as Topic/
13.	comment/
14.	case report/
15.	(letter or comment*).ti.
16.	or/8-15
17.	randomized controlled trial/ or random*.ti,ab.
18.	16 not 17
19.	animals/ not humans/
20.	exp Animals, Laboratory/
21.	exp Animal Experimentation/
22.	exp Models, Animal/
23.	exp Rodentia/
24.	(rat or rats or mouse or mice or rodent*).ti.
25.	or/18-24

26.	7 not 25
27.	limit 26 to English language
28.	exp Vitamin B 12/
29.	Transcobalamins/
30.	Methylmalonic Acid/
31.	Homocysteine/
32.	((total or active or serum or vitamin) adj4 (b12 or b 12 or cobalamin or cbl)).ti,ab,kf.
33.	(holotranscobalamin or methylmalonic or homocysteine).ti,ab,kf.
34.	(holoTC or MMA or Hcy).ti,ab.
35.	or/28-34
36.	exp Diagnosis/
37.	(diagnos* or test* or assay* or analys* or analyz* or measur* or marker* or biomarker* or immunoassay* or indicator* or detect* or evaluat* or screen*).ti,ab,kf.
38.	or/36-37
39.	35 and 38
40.	27 and 39
41.	randomized controlled trial.pt.
42.	controlled clinical trial.pt.
43.	randomi#ed.ab.
44.	placebo.ab.
45.	randomly.ab.
46.	clinical trials as topic.sh.
47.	trial.ti.
48.	or/41-47
49.	40 not 48

1 **Embase (Ovid) search terms**

1.	exp B12 deficiency/
2.	((b12 or b 12 or cobalamin* or c?anocobalamin* or transcobalamin*) adj4 (deficien* or malabsor* or absor* or lack* or diminish* or low* or level* or abnormal* or deficit or disorder* or inadequa* or hypovitaminosis or hypo vitaminosis or avitaminosis)).ti,ab.
3.	exp macrocytic anemia/
4.	((b12 or b 12 or macrocytic or megaloblastic or pernicious or addison*) adj3 (anemia* or anaemia*)).ti,ab.
5.	intrinsic factor/
6.	intrinsic factor.ti,ab.
7.	or/1-6
8.	letter.pt. or letter/
9.	note.pt.
10.	editorial.pt.
11.	case report/ or case study/
12.	(letter or comment*).ti.
13.	(conference abstract or conference paper).pt.
14.	or/8-13
15.	randomized controlled trial/ or random*.ti,ab.
16.	14 not 15
17.	animal/ not human/
18.	nonhuman/
19.	exp Animal Experiment/

20.	exp Experimental Animal/
21.	animal model/
22.	exp Rodent/
23.	(rat or rats or mouse or mice or rodent*).ti.
24.	or/16-23
25.	7 not 24
26.	limit 25 to English language
27.	cyanocobalamin/
28.	transcobalamin/
29.	methylmalonic acid/
30.	homocysteine/
31.	((total or active or serum or vitamin) adj4 (b12 or b 12 or cobalamin or cbl)).ti,ab,kf.
32.	(holotranscobalamin or methylmalonic or homocysteine).ti,ab,kf.
33.	(holoTC or MMA or Hcy).ti,ab.
34.	or/27-33
35.	diagnostic procedure/
36.	diagnosis/
37.	(diagnos* or test* or assay* or analys* or analyz* or measur* or marker* or biomarker* or immunoassay* or indicator* or detect* or evaluat* or screen*).ti,ab,kf.
38.	or/35-37
39.	34 and 38
40.	26 and 39
41.	random*.ti,ab.
42.	factorial*.ti,ab.
43.	(crossover* or cross over*).ti,ab.
44.	((doubl* or singl*) adj blind*).ti,ab.
45.	(assign* or allocat* or volunteer* or placebo*).ti,ab.
46.	crossover procedure/
47.	single blind procedure/
48.	randomized controlled trial/
49.	double blind procedure/
50.	or/41-49
51.	40 not 50

1 **Cochrane Library (Wiley) search terms**

#1.	MeSH descriptor: [Vitamin B 12 Deficiency] explode all trees
#2.	((b12 or b 12 or cobalamin* or c?anocobalamin* or transcobalamin*) near/4 (deficien* or malabsor* or absor* or lack* or diminish* or low* or level* or abnormal* or deficit or disorder* or inadequa* or hypovitaminosis or hypo vitaminosis or avitaminosis)):ti,ab
#3.	MeSH descriptor: [Anemia, Macrocytic] explode all trees
#4.	((b12 or b 12 or macrocytic or megaloblastic or pernicious or addison*) near/3 (anemia* or anaemia*)):ti,ab
#5.	MeSH descriptor: [Intrinsic Factor] this term only
#6.	intrinsic factor:ti,ab
#7.	(or #1-#6)
#8.	conference:pt or (clinicaltrials or trialsearch):so
#9.	#7 not #8
#10.	MeSH descriptor: [Vitamin B 12] explode all trees
#11.	MeSH descriptor: [Transcobalamins] this term only

#12.	MeSH descriptor: [Methylmalonic Acid] this term only
#13.	MeSH descriptor: [Homocysteine] this term only
#14.	MeSH descriptor: [Hematologic Tests] this term only
#15.	((total or active or serum or vitamin) near/4 (b12 or b 12 or cobalamin or cbl)):ti,ab,kw
#16.	(holotranscobalamin or methylmalonic or homocysteine):ti,ab,kw
#17.	(holoTC or MMA or Hcy):ti,ab
#18.	(or #10-#17)
#19.	MeSH descriptor: [Diagnosis] explode all trees
#20.	(diagnos* or test* or assay* or analys* or analyz* or measur* or marker* or biomarker* or immunoassay* or indicator* or detect* or evaluat* or screen*):ti,ab,kw
#21.	(or #19-#20)
#22.	#18 and #21
#23.	#9 and #22

Epistemonikos search terms

1.	(title:("b12 deficien*" OR "B 12 deficien*" OR "cobalamin* deficien*" OR "c?anocobalamin* deficien*" OR "transcobalamin* deficien*" OR "b12 malabsor*" OR "b 12 malabsor*" OR "cobalamin* malabsor*" OR "c?anocobalamin* malabsor*" OR "transcobalamin* malabsor*" OR "b12 anemia*" OR "b 12 anemia*" OR "macrocytic anemia*" OR "megaloblastic anemia*" OR "pernicious anemia*" OR "addison* anemia*" OR "b12 anaemia*" OR "b 12 anaemia*" OR "macrocytic anaemia*" OR "megaloblastic anaemia*" OR "pernicious anaemia*" OR "addison* anaemia*" OR "intrinsic factor") OR abstract:("b12 deficien*" OR "B 12 deficien*" OR "cobalamin* deficien*" OR "c?anocobalamin* deficien*" OR "transcobalamin* deficien*" OR "b12 malabsor*" OR "b 12 malabsor*" OR "cobalamin* malabsor*" OR "c?anocobalamin* malabsor*" OR "transcobalamin* malabsor*" OR "b12 anemia*" OR "b 12 anemia*" OR "macrocytic anemia*" OR "megaloblastic anemia*" OR "pernicious anemia*" OR "addison* anemia*" OR "b12 anaemia*" OR "b 12 anaemia*" OR "macrocytic anaemia*" OR "megaloblastic anaemia*" OR "pernicious anaemia*" OR "addison* anaemia*" OR "intrinsic factor")) AND (title:("serum b12" OR "serum b 12" OR "serum cobalamin" OR "serum cbl" OR "total b12" OR "total b 12" OR "total cobalamin" OR "total cbl" OR "active b12" OR "active b 12" OR "active cobalamin" OR "active cbl" OR "vitamin b12" OR "vitamin b 12" OR "vitamin cobalamin" OR "vitamin cbl" OR holotranscobalamin OR methylmalonic OR homocysteine OR holoTC OR MMA OR Hcy) OR abstract:("serum b12" OR "serum b 12" OR "serum cobalamin" OR "serum cbl" OR "total b12" OR "total b 12" OR "total cobalamin" OR "total cbl" OR "active b12" OR "active b 12" OR "active cobalamin" OR "active cbl" OR "vitamin b12" OR "vitamin b 12" OR "vitamin cobalamin" OR "vitamin cbl" OR holotranscobalamin OR methylmalonic OR homocysteine OR holoTC OR MMA OR Hcy)) AND (title:(diagnos* OR test* OR assay* OR analys* OR analyz* OR measur* OR marker* OR biomarker* OR immunoassay* OR indicator* OR detect* OR evaluat* OR screen*) OR abstract:(diagnos* OR test* OR assay* OR analys* OR analyz* OR measur* OR marker* OR biomarker* OR immunoassay* OR indicator* OR detect* OR evaluat* OR screen*)

B.1.2 Health Economics literature search strategy

Health economic evidence was identified by conducting searches using terms for a broad Vitamin B12 deficient population. The following databases were searched: NHS Economic Evaluation Database (NHS EED - this ceased to be updated after 31st March 2015), Health Technology Assessment database (HTA - this ceased to be updated from 31st March 2018) and The International Network of Agencies for Health Technology Assessment (INAHTA). Searches for recent evidence were run on Medline and Embase from 2014 onwards for health economics, and all years for quality-of-life studies.

Table 18: Database parameters, filters and limits applied

Database	Dates searched	Search filters and limits applied
Medline (OVID)	Health Economics 1 January 2014 – 16 December 2022	Health economics studies Quality of life studies Exclusions (animal studies, letters, comments, editorials, case studies/reports) English language
	Quality of Life 1946 – 16 December 2022	
Embase (OVID)	Health Economics 1 January 2014 – 16 December 2022	Health economics studies Quality of life studies Exclusions (animal studies, letters, comments, editorials, case studies/reports, conference abstracts) English language
	Quality of Life 1974 – 16 December 2022	
NHS Economic Evaluation Database (NHS EED) (Centre for Research and Dissemination - CRD)	Inception –31 March 2015	
Health Technology Assessment Database (HTA) (Centre for Research and Dissemination – CRD)	Inception – 31 March 2018	
The International Network of Agencies for Health Technology Assessment (INAHTA)	Inception - 16 December 2022	English language

Medline (Ovid) search terms

1.	exp Vitamin B 12 Deficiency/
2.	((b12 or b 12 or cobalamin* or c?anocobalamin* or transcobalamin*) adj4 (deficien* or malabsor* or absor* or lack* or diminish* or low* or level* or abnormal* or deficit or disorder* or inadequa* or hypovitaminosis or hypo vitaminosis or avitaminosis)).ti,ab.
3.	exp Macrocytic Anemia/
4.	((b12 or b 12 or macrocytic or megaloblastic or pernicious or addison*) adj3 (anemia* or anaemia*)).ti,ab.
5.	Intrinsic Factor/
6.	intrinsic factor.ti,ab.
7.	or/1-6
8.	letter/
9.	editorial/
10.	news/
11.	exp historical article/
12.	Anecdotes as Topic/
13.	comment/

14.	case report/
15.	(letter or comment*).ti.
16.	or/8-15
17.	randomized controlled trial/ or random*.ti,ab.
18.	16 not 17
19.	animals/ not humans/
20.	exp Animals, Laboratory/
21.	exp Animal Experimentation/
22.	exp Models, Animal/
23.	exp Rodentia/
24.	(rat or rats or mouse or mice or rodent*).ti.
25.	or/18-24
26.	7 not 25
27.	limit 26 to English language
28.	quality-adjusted life years/
29.	sickness impact profile/
30.	(quality adj2 (wellbeing or well being)).ti,ab.
31.	sickness impact profile.ti,ab.
32.	disability adjusted life.ti,ab.
33.	(qal* or qtime* or qwb* or daly*).ti,ab.
34.	(euroqol* or eq5d* or eq 5*).ti,ab.
35.	(qol* or hql* or hqol* or h qol* or hrqol* or hr qol*).ti,ab.
36.	(health utility* or utility score* or disutilit* or utility value*).ti,ab.
37.	(hui or hui1 or hui2 or hui3).ti,ab.
38.	(health* year* equivalent* or hye or hyes).ti,ab.
39.	discrete choice*.ti,ab.
40.	rosser.ti,ab.
41.	(willingness to pay or time tradeoff or time trade off or tto or standard gamble*).ti,ab.
42.	(sf36* or sf 36* or short form 36* or shortform 36* or shortform36*).ti,ab.
43.	(sf20 or sf 20 or short form 20 or shortform 20 or shortform20).ti,ab.
44.	(sf12* or sf 12* or short form 12* or shortform 12* or shortform12*).ti,ab.
45.	(sf8* or sf 8* or short form 8* or shortform 8* or shortform8*).ti,ab.
46.	(sf6* or sf 6* or short form 6* or shortform 6* or shortform6*).ti,ab.
47.	or/28-46
48.	Economics/
49.	Value of life/
50.	exp "Costs and Cost Analysis"/
51.	exp Economics, Hospital/
52.	exp Economics, Medical/
53.	Economics, Nursing/
54.	Economics, Pharmaceutical/
55.	exp "Fees and Charges"/

56.	exp Budgets/
57.	budget*.ti,ab.
58.	cost*.ti.
59.	(economic* or pharmaco?economic*).ti.
60.	(price* or pricing*).ti,ab.
61.	(cost* adj2 (effective* or utilit* or benefit* or minimi* or unit* or estimat* or variable*)).ab.
62.	(financ* or fee or fees).ti,ab.
63.	(value adj2 (money or monetary)).ti,ab.
64.	or/48-63
65.	27 and 47
66.	27 and 64
67.	limit 66 to yr="2014 -Current"
68.	65 or 67

1 **Embase (Ovid) search terms**

1.	exp B12 deficiency/
2.	((b12 or b 12 or cobalamin* or c?anocobalamin* or transcobalamin*) adj4 (deficien* or malabsor* or absor* or lack* or diminish* or low* or level* or abnormal* or deficit or disorder* or inadequa* or hypovitaminosis or hypo vitaminosis or avitaminosis)).ti,ab.
3.	exp macrocytic anemia/
4.	((b12 or b 12 or macrocytic or megaloblastic or pernicious or addison*) adj3 (anemia* or anaemia*)).ti,ab.
5.	intrinsic factor/
6.	intrinsic factor.ti,ab.
7.	or/1-6
8.	letter.pt. or letter/
9.	note.pt.
10.	editorial.pt.
11.	case report/ or case study/
12.	(letter or comment*).ti.
13.	(conference abstract or conference paper).pt.
14.	or/8-13
15.	randomized controlled trial/ or random*.ti,ab.
16.	14 not 15
17.	animal/ not human/
18.	nonhuman/
19.	exp Animal Experiment/
20.	exp Experimental Animal/
21.	animal model/
22.	exp Rodent/
23.	(rat or rats or mouse or mice or rodent*).ti.
24.	or/16-23
25.	7 not 24
26.	limit 25 to English language
27.	quality adjusted life year/

28.	"quality of life index"/
29.	short form 12/ or short form 20/ or short form 36/ or short form 8/
30.	sickness impact profile/
31.	(quality adj2 (wellbeing or well being)).ti,ab.
32.	sickness impact profile.ti,ab.
33.	disability adjusted life.ti,ab.
34.	(qal* or qtime* or qwb* or daly*).ti,ab.
35.	(euroqol* or eq5d* or eq 5*).ti,ab.
36.	(qol* or hql* or hqol* or h qol* or hrqol* or hr qol*).ti,ab.
37.	(health utility* or utility score* or disutilit* or utility value*).ti,ab.
38.	(hui or hui1 or hui2 or hui3).ti,ab.
39.	(health* year* equivalent* or hye or hyes).ti,ab.
40.	discrete choice*.ti,ab.
41.	rosser.ti,ab.
42.	(willingness to pay or time tradeoff or time trade off or tto or standard gamble*).ti,ab.
43.	(sf36* or sf 36* or short form 36* or shortform 36* or shortform36*).ti,ab.
44.	(sf20 or sf 20 or short form 20 or shortform 20 or shortform20).ti,ab.
45.	(sf12* or sf 12* or short form 12* or shortform 12* or shortform12*).ti,ab.
46.	(sf8* or sf 8* or short form 8* or shortform 8* or shortform8*).ti,ab.
47.	(sf6* or sf 6* or short form 6* or shortform 6* or shortform6*).ti,ab.
48.	or/27-47
49.	health economics/
50.	exp economic evaluation/
51.	exp health care cost/
52.	exp fee/
53.	budget/
54.	funding/
55.	budget*.ti,ab.
56.	cost*.ti.
57.	(economic* or pharmaco?economic*).ti.
58.	(price* or pricing*).ti,ab.
59.	(cost* adj2 (effective* or utilit* or benefit* or minimi* or unit* or estimat* or variable*)).ab.
60.	(financ* or fee or fees).ti,ab.
61.	(value adj2 (money or monetary)).ti,ab.
62.	or/49-61
63.	26 and 48
64.	26 and 62
65.	limit 64 to yr="2014 -Current"
66.	63 or 65

1 **NHS EED and HTA (CRD) search terms**

#1.	MeSH DESCRIPTOR Vitamin B 12 Deficiency EXPLODE ALL TREES
#2.	MeSH DESCRIPTOR Anemia, Macrocytic EXPLODE ALL TREES
#3.	((b12 or b 12 or cobalamin* or c?anocobalamin* or transcobalamin*) adj4 (deficien* or malabsor* or absor* or lack* or diminish* or low* or level* or abnormal* or deficit or disorder* or inadequa* or hypovitaminosis or hypo vitaminosis or avitaminosis))
#4.	((b12 or b 12 or macrocytic or megaloblastic or pernicious or addison*) adj3 (anemia* or anaemia*))

#5.	(intrinsic factor)
#6.	#1 OR #2 OR #3 OR #4 OR #5

INAHTA search terms

1.	(Anemia, Pernicious)[mh] OR (Vitamin B 12 Deficiency)[mh] OR (pernicious anemia) OR (pernicious anemia) OR (B12) OR (B 12)

B.2 What are the most clinically and cost-effective ways to diagnose vitamin B12 deficiency, including the serum cobalamin assay and holotranscobalamin, methylmalonic acid and homocysteine tests?

B.2.1 Clinical search literature search strategy

Searches were constructed using a PICO framework where population (P) terms were combined with Intervention (I) and in some cases Comparison (C) terms. Outcomes (O) are rarely used in search strategies as these concepts may not be indexed or described in the title or abstract and are therefore difficult to retrieve. Search filters were applied to the search where appropriate.

Table 19: Database parameters, filters and limits applied

Database	Dates searched	Search filter used
Medline (OVID)	1946 – 16 December 2022	Randomised controlled trials Systematic review studies Exclusions (animal studies, letters, comments, editorials, case studies/reports) English language
Embase (OVID)	1974 – 16 December 2022	Randomised controlled trials Systematic review studies Exclusions (animal studies, letters, comments, editorials, case studies/reports, conference abstracts) English language
The Cochrane Library (Wiley)	Cochrane Database of Systematic Reviews to Issue 12 of 12, 16 December 2022 Cochrane Central Register of Controlled Trials to Issue 12 of 12, 16 December 2022	Exclusions (clinical trials, conference abstracts)

Database	Dates searched	Search filter used
Epistemonikos (The Epistemonikos Foundation)	Inception to 16 December 2022	Systematic review Exclusions (Cochrane reviews)

1 **Medline (Ovid) search terms**

1.	exp Vitamin B 12 Deficiency/
2.	((b12 or b 12 or cobalamin* or c?anocobalamin* or transcobalamin*) adj4 (deficien* or malabsor* or absor* or lack* or diminish* or low* or level* or abnormal* or deficit or disorder* or inadequa* or hypovitaminosis or hypo vitaminosis or avitaminosis)).ti,ab.
3.	exp Macrocytic Anemia/
4.	((b12 or b 12 or macrocytic or megaloblastic or pernicious or addison*) adj3 (anemia* or anaemia*)).ti,ab.
5.	Intrinsic Factor/
6.	intrinsic factor.ti,ab.
7.	or/1-6
8.	letter/
9.	editorial/
10.	news/
11.	exp historical article/
12.	Anecdotes as Topic/
13.	comment/
14.	case report/
15.	(letter or comment*).ti.
16.	or/8-15
17.	randomized controlled trial/ or random*.ti,ab.
18.	16 not 17
19.	animals/ not humans/
20.	exp Animals, Laboratory/
21.	exp Animal Experimentation/
22.	exp Models, Animal/
23.	exp Rodentia/
24.	(rat or rats or mouse or mice or rodent*).ti.
25.	or/18-24
26.	7 not 25
27.	limit 26 to English language
28.	exp Vitamin B 12/
29.	Transcobalamins/
30.	Methylmalonic Acid/
31.	Homocysteine/
32.	((total or active or serum or vitamin) adj4 (b12 or b 12 or cobalamin or cbl)).ti,ab,kf.
33.	(holotranscobalamin or methylmalonic or homocysteine).ti,ab,kf.
34.	(holoTC or MMA or Hcy).ti,ab.
35.	or/28-34
36.	exp Diagnosis/
37.	(diagnos* or test* or assay* or analys* or analyz* or measur* or marker* or biomarker* or immunoassay* or indicator* or detect* or evaluat* or screen*).ti,ab,kf.
38.	or/36-37
39.	35 and 38

40.	27 and 39
41.	Meta-Analysis/
42.	Meta-Analysis as Topic/
43.	(meta analy* or metanaly* or metaanaly* or meta regression).ti,ab.
44.	((systematic* or evidence*) adj3 (review* or overview*)).ti,ab.
45.	(reference list* or bibliograph* or hand search* or manual search* or relevant journals).ab.
46.	(search strategy or search criteria or systematic search or study selection or data extraction).ab.
47.	(search* adj4 literature).ab.
48.	(medline or pubmed or cochrane or embase or psychlit or psyclit or psychinfo or psycinfo or cinahl or science citation index or bids or cancerlit).ab.
49.	cochrane.jw.
50.	((multiple treatment* or indirect or mixed) adj2 comparison*).ti,ab.
51.	or/41-50
52.	randomized controlled trial.pt.
53.	controlled clinical trial.pt.
54.	randomi#ed.ab.
55.	placebo.ab.
56.	randomly.ab.
57.	clinical trials as topic.sh.
58.	trial.ti.
59.	or/52-58
60.	40 and (51 or 59)

1 **Embase (Ovid) search terms**

1.	exp B12 deficiency/
2.	((b12 or b 12 or cobalamin* or c?anocobalamin* or transcobalamin*) adj4 (deficien* or malabsor* or absor* or lack* or diminish* or low* or level* or abnormal* or deficit or disorder* or inadequa* or hypovitaminosis or hypo vitaminosis or avitaminosis)).ti,ab.
3.	exp macrocytic anemia/
4.	((b12 or b 12 or macrocytic or megaloblastic or pernicious or addison*) adj3 (anemia* or anaemia*)).ti,ab.
5.	intrinsic factor/
6.	intrinsic factor.ti,ab.
7.	or/1-6
8.	letter.pt. or letter/
9.	note.pt.
10.	editorial.pt.
11.	case report/ or case study/
12.	(letter or comment*).ti.
13.	(conference abstract or conference paper).pt.
14.	or/8-13
15.	randomized controlled trial/ or random*.ti,ab.
16.	14 not 15
17.	animal/ not human/
18.	nonhuman/
19.	exp Animal Experiment/
20.	exp Experimental Animal/

21.	animal model/
22.	exp Rodent/
23.	(rat or rats or mouse or mice or rodent*).ti.
24.	or/16-23
25.	7 not 24
26.	limit 25 to English language
27.	cyanocobalamin/
28.	transcobalamin/
29.	methylmalonic acid/
30.	homocysteine/
31.	((total or active or serum or vitamin) adj4 (b12 or b 12 or cobalamin or cbl)).ti,ab,kf.
32.	(holotranscobalamin or methylmalonic or homocysteine).ti,ab,kf.
33.	(holoTC or MMA or Hcy).ti,ab.
34.	or/27-33
35.	diagnostic procedure/
36.	diagnosis/
37.	(diagnos* or test* or assay* or analys* or analyz* or measur* or marker* or biomarker* or immunoassay* or indicator* or detect* or evaluat* or screen*).ti,ab,kf.
38.	or/35-37
39.	34 and 38
40.	26 and 39
41.	random*.ti,ab.
42.	factorial*.ti,ab.
43.	(crossover* or cross over*).ti,ab.
44.	((doubl* or singl*) adj blind*).ti,ab.
45.	(assign* or allocat* or volunteer* or placebo*).ti,ab.
46.	crossover procedure/
47.	single blind procedure/
48.	randomized controlled trial/
49.	double blind procedure/
50.	or/41-49
51.	Systematic Review/
52.	Meta-Analysis/
53.	(meta analy* or metanaly* or metaanaly* or meta regression).ti,ab.
54.	((systematic* or evidence*) adj3 (review* or overview*)).ti,ab.
55.	(reference list* or bibliograph* or hand search* or manual search* or relevant journals).ab.
56.	(search strategy or search criteria or systematic search or study selection or data extraction).ab.
57.	(search* adj4 literature).ab.
58.	(medline or pubmed or cochrane or embase or psychlit or psyclit or psychinfo or psycinfo or cinahl or science citation index or bids or cancerlit).ab.
59.	cochrane.jw.
60.	((multiple treatment* or indirect or mixed) adj2 comparison*).ti,ab.
61.	or/51-60
62.	40 and (50 or 61)

1 **Cochrane Library (Wiley) search terms**

#1.	MeSH descriptor: [Vitamin B 12 Deficiency] explode all trees

#2.	((b12 or b 12 or cobalamin* or c?anocobalamin* or transcobalamin*) near/4 (deficien* or malabsor* or absor* or lack* or diminish* or low* or level* or abnormal* or deficit or disorder* or inadequa* or hypovitaminosis or hypo vitaminosis or avitaminosis)):ti,ab
#3.	MeSH descriptor: [Anemia, Macrocytic] explode all trees
#4.	((b12 or b 12 or macrocytic or megaloblastic or pernicious or addison*) near/3 (anemia* or anaemia*)):ti,ab
#5.	MeSH descriptor: [Intrinsic Factor] this term only
#6.	intrinsic factor:ti,ab
#7.	(or #1-#6)
#8.	conference:pt or (clinicaltrials or trialsearch):so
#9.	#7 not #8
#10.	MeSH descriptor: [Vitamin B 12] explode all trees
#11.	MeSH descriptor: [Transcobalamins] this term only
#12.	MeSH descriptor: [Methylmalonic Acid] this term only
#13.	MeSH descriptor: [Homocysteine] this term only
#14.	MeSH descriptor: [Hematologic Tests] this term only
#15.	((total or active or serum or vitamin) near/4 (b12 or b 12 or cobalamin or cbl)):ti,ab,kw
#16.	(holotranscobalamin or methylmalonic or homocysteine):ti,ab,kw
#17.	(holoTC or MMA or Hcy):ti,ab
#18.	(or #10-#17)
#19.	MeSH descriptor: [Diagnosis] explode all trees
#20.	(diagnos* or test* or assay* or analys* or analyz* or measur* or marker* or biomarker* or immunoassay* or indicator* or detect* or evaluat* or screen*):ti,ab,kw
#21.	(or #19-#20)
#22.	#18 and #21
#23.	#9 and #22

1 **Epistemonikos search terms**

1.	(title:("b12 deficien*" OR "B 12 deficien*" OR "cobalamin* deficien*" OR "c?anocobalamin* deficien*" OR "transcobalamin* deficien*" OR "b12 malabsor*" OR "b 12 malabsor*" OR "cobalamin* malabsor*" OR "c?anocobalamin* malabsor*" OR "transcobalamin* malabsor*" OR "b12 anemia*" OR "b 12 anemia*" OR "macrocytic anemia*" OR "megaloblastic anemia*" OR "pernicious anemia*" OR "addison* anemia*" OR "b12 anaemia*" OR "b 12 anaemia*" OR "macrocytic anaemia*" OR "megaloblastic anaemia*" OR "pernicious anaemia*" OR "addison* anaemia*" OR "intrinsic factor") OR abstract:("b12 deficien*" OR "B 12 deficien*" OR "cobalamin* deficien*" OR "c?anocobalamin* deficien*" OR "transcobalamin* deficien*" OR "b12 malabsor*" OR "b 12 malabsor*" OR "cobalamin* malabsor*" OR "c?anocobalamin* malabsor*" OR "transcobalamin* malabsor*" OR "b12 anemia*" OR "b 12 anemia*" OR "macrocytic anemia*" OR "megaloblastic anemia*" OR "pernicious anemia*" OR "addison* anemia*" OR "b12 anaemia*" OR "b 12 anaemia*" OR "macrocytic anaemia*" OR "megaloblastic anaemia*" OR "pernicious anaemia*" OR "addison* anaemia*" OR "intrinsic factor")) AND (title:("serum b12" OR "serum b 12" OR "serum cobalamin" OR "serum cbl" OR "total b12" OR "total b 12" OR "total cobalamin" OR "total cbl" OR "active b12" OR "active b 12" OR "active cobalamin" OR "active cbl" OR "vitamin b12" OR "vitamin b 12" OR "vitamin cobalamin" OR "vitamin cbl" OR holotranscobalamin OR methylmalonic OR homocysteine OR holoTC OR MMA OR Hcy) OR abstract:("serum b12" OR "serum b 12" OR "serum cobalamin" OR "serum cbl" OR "total b12" OR "total b 12" OR "total cobalamin" OR "total cbl" OR "active b12" OR "active b 12" OR "active cobalamin" OR "active cbl" OR "vitamin b12" OR "vitamin b 12" OR "vitamin cobalamin" OR "vitamin cbl" OR holotranscobalamin OR methylmalonic OR homocysteine OR holoTC OR MMA OR Hcy)) AND (title:(diagnos* OR test* OR assay* OR analys* OR analyz* OR measur* OR marker* OR biomarker* OR immunoassay* OR indicator* OR detect* OR evaluat* OR screen*) OR abstract:(diagnos* OR test* OR assay* OR analys* OR analyz* OR measur* OR

	marker* OR biomarker* OR immunoassay* OR indicator* OR detect* OR evaluat* OR screen*)

B.2.21 Health Economics literature search strategy

Health economic evidence was identified by conducting searches using terms for a broad Vitamin B12 deficient population. The following databases were searched: NHS Economic Evaluation Database (NHS EED - this ceased to be updated after 31st March 2015), Health Technology Assessment database (HTA - this ceased to be updated from 31st March 2018) and The International Network of Agencies for Health Technology Assessment (INAHTA). Searches for recent evidence were run on Medline and Embase from 2014 onwards for health economics, and all years for quality-of-life studies.

Table 20: Database parameters, filters and limits applied

Database	Dates searched	Search filters and limits applied
Medline (OVID)	Health Economics 1 January 2014 – 16 December 2022	Health economics studies Quality of life studies
	Quality of Life 1946 – 16 December 2022	Exclusions (animal studies, letters, comments, editorials, case studies/reports) English language
Embase (OVID)	Health Economics 1 January 2014 – 16 December 2022	Health economics studies Quality of life studies
	Quality of Life 1974 – 16 December 2022	Exclusions (animal studies, letters, comments, editorials, case studies/reports, conference abstracts) English language
NHS Economic Evaluation Database (NHS EED) (Centre for Research and Dissemination - CRD)	Inception –31 March 2015	
Health Technology Assessment Database (HTA) (Centre for Research and Dissemination – CRD)	Inception – 31 March 2018	
The International Network of Agencies for Health Technology Assessment (INAHTA)	Inception - 16 December 2022	English language

Medline (Ovid) search terms

1.	exp Vitamin B 12 Deficiency/
2.	((b12 or b 12 or cobalamin* or c?anocobalamin* or transcobalamin*) adj4 (deficien* or malabsor* or absor* or lack* or diminish* or low* or level* or abnormal* or deficit or disorder* or inadequa* or hypovitaminosis or hypo vitaminosis or avitaminosis)).ti,ab.
3.	exp Macrocytic Anemia/

4.	((b12 or b 12 or macrocytic or megaloblastic or pernicious or addison*) adj3 (anemia* or anaemia*)).ti,ab.
5.	Intrinsic Factor/
6.	intrinsic factor.ti,ab.
7.	or/1-6
8.	letter/
9.	editorial/
10.	news/
11.	exp historical article/
12.	Anecdotes as Topic/
13.	comment/
14.	case report/
15.	(letter or comment*).ti.
16.	or/8-15
17.	randomized controlled trial/ or random*.ti,ab.
18.	16 not 17
19.	animals/ not humans/
20.	exp Animals, Laboratory/
21.	exp Animal Experimentation/
22.	exp Models, Animal/
23.	exp Rodentia/
24.	(rat or rats or mouse or mice or rodent*).ti.
25.	or/18-24
26.	7 not 25
27.	limit 26 to English language
28.	quality-adjusted life years/
29.	sickness impact profile/
30.	(quality adj2 (wellbeing or well being)).ti,ab.
31.	sickness impact profile.ti,ab.
32.	disability adjusted life.ti,ab.
33.	(qal* or qtime* or qwb* or daly*).ti,ab.
34.	(euroqol* or eq5d* or eq 5*).ti,ab.
35.	(qol* or hql* or hqol* or h qol* or hrqol* or hr qol*).ti,ab.
36.	(health utility* or utility score* or disutilit* or utility value*).ti,ab.
37.	(hui or hui1 or hui2 or hui3).ti,ab.
38.	(health* year* equivalent* or hye or hyes).ti,ab.
39.	discrete choice*.ti,ab.
40.	rosser.ti,ab.
41.	(willingness to pay or time tradeoff or time trade off or tto or standard gamble*).ti,ab.
42.	(sf36* or sf 36* or short form 36* or shortform 36* or shortform36*).ti,ab.
43.	(sf20 or sf 20 or short form 20 or shortform 20 or shortform20).ti,ab.
44.	(sf12* or sf 12* or short form 12* or shortform 12* or shortform12*).ti,ab.
45.	(sf8* or sf 8* or short form 8* or shortform 8* or shortform8*).ti,ab.

46.	(sf6* or sf 6* or short form 6* or shortform 6* or shortform6*).ti,ab.
47.	or/28-46
48.	Economics/
49.	Value of life/
50.	exp "Costs and Cost Analysis"/
51.	exp Economics, Hospital/
52.	exp Economics, Medical/
53.	Economics, Nursing/
54.	Economics, Pharmaceutical/
55.	exp "Fees and Charges"/
56.	exp Budgets/
57.	budget*.ti,ab.
58.	cost*.ti.
59.	(economic* or pharmaco?economic*).ti.
60.	(price* or pricing*).ti,ab.
61.	(cost* adj2 (effective* or utilit* or benefit* or minimi* or unit* or estimat* or variable*)).ab.
62.	(financ* or fee or fees).ti,ab.
63.	(value adj2 (money or monetary)).ti,ab.
64.	or/48-63
65.	27 and 47
66.	27 and 64
67.	limit 66 to yr="2014 -Current"
68.	65 or 67

1 **Embase (Ovid) search terms**

1.	exp B12 deficiency/
2.	((b12 or b 12 or cobalamin* or c?anocobalamin* or transcobalamin*) adj4 (deficien* or malabsor* or absor* or lack* or diminish* or low* or level* or abnormal* or deficit or disorder* or inadequa* or hypovitaminosis or hypo vitaminosis or avitaminosis)).ti,ab.
3.	exp macrocytic anemia/
4.	((b12 or b 12 or macrocytic or megaloblastic or pernicious or addison*) adj3 (anemia* or anaemia*)).ti,ab.
5.	intrinsic factor/
6.	intrinsic factor.ti,ab.
7.	or/1-6
8.	letter.pt. or letter/
9.	note.pt.
10.	editorial.pt.
11.	case report/ or case study/
12.	(letter or comment*).ti.
13.	(conference abstract or conference paper).pt.
14.	or/8-13
15.	randomized controlled trial/ or random*.ti,ab.
16.	14 not 15

17.	animal/ not human/
18.	nonhuman/
19.	exp Animal Experiment/
20.	exp Experimental Animal/
21.	animal model/
22.	exp Rodent/
23.	(rat or rats or mouse or mice or rodent*).ti.
24.	or/16-23
25.	7 not 24
26.	limit 25 to English language
27.	quality adjusted life year/
28.	"quality of life index"/
29.	short form 12/ or short form 20/ or short form 36/ or short form 8/
30.	sickness impact profile/
31.	(quality adj2 (wellbeing or well being)).ti,ab.
32.	sickness impact profile.ti,ab.
33.	disability adjusted life.ti,ab.
34.	(qal* or qtime* or qwb* or daly*).ti,ab.
35.	(euroqol* or eq5d* or eq 5*).ti,ab.
36.	(qol* or hql* or hqol* or h qol* or hrqol* or hr qol*).ti,ab.
37.	(health utility* or utility score* or disutilit* or utility value*).ti,ab.
38.	(hui or hui1 or hui2 or hui3).ti,ab.
39.	(health* year* equivalent* or hye or hyes).ti,ab.
40.	discrete choice*.ti,ab.
41.	rosser.ti,ab.
42.	(willingness to pay or time tradeoff or time trade off or tto or standard gamble*).ti,ab.
43.	(sf36* or sf 36* or short form 36* or shortform 36* or shortform36*).ti,ab.
44.	(sf20 or sf 20 or short form 20 or shortform 20 or shortform20).ti,ab.
45.	(sf12* or sf 12* or short form 12* or shortform 12* or shortform12*).ti,ab.
46.	(sf8* or sf 8* or short form 8* or shortform 8* or shortform8*).ti,ab.
47.	(sf6* or sf 6* or short form 6* or shortform 6* or shortform6*).ti,ab.
48.	or/27-47
49.	health economics/
50.	exp economic evaluation/
51.	exp health care cost/
52.	exp fee/
53.	budget/
54.	funding/
55.	budget*.ti,ab.
56.	cost*.ti.
57.	(economic* or pharmaco?economic*).ti.
58.	(price* or pricing*).ti,ab.
59.	(cost* adj2 (effective* or utilit* or benefit* or minimi* or unit* or estimat* or variable*)).ab.
60.	(financ* or fee or fees).ti,ab.
61.	(value adj2 (money or monetary)).ti,ab.
62.	or/49-61

63.	26 and 48
64.	26 and 62
65.	limit 64 to yr="2014 -Current"
66.	63 or 65

NHS EED and HTA (CRD) search terms

#1.	MeSH DESCRIPTOR Vitamin B 12 Deficiency EXPLODE ALL TREES
#2.	MeSH DESCRIPTOR Anemia, Macrocytic EXPLODE ALL TREES
#3.	((b12 or b 12 or cobalamin* or c?anocobalamin* or transcobalamin*) adj4 (deficien* or malabsor* or absor* or lack* or diminish* or low* or level* or abnormal* or deficit or disorder* or inadequa* or hypovitaminosis or hypo vitaminosis or avitaminosis))
#4.	((b12 or b 12 or macrocytic or megaloblastic or pernicious or addison*) adj3 (anemia* or anaemia*))
#5.	(intrinsic factor)
#6.	#1 OR #2 OR #3 OR #4 OR #5

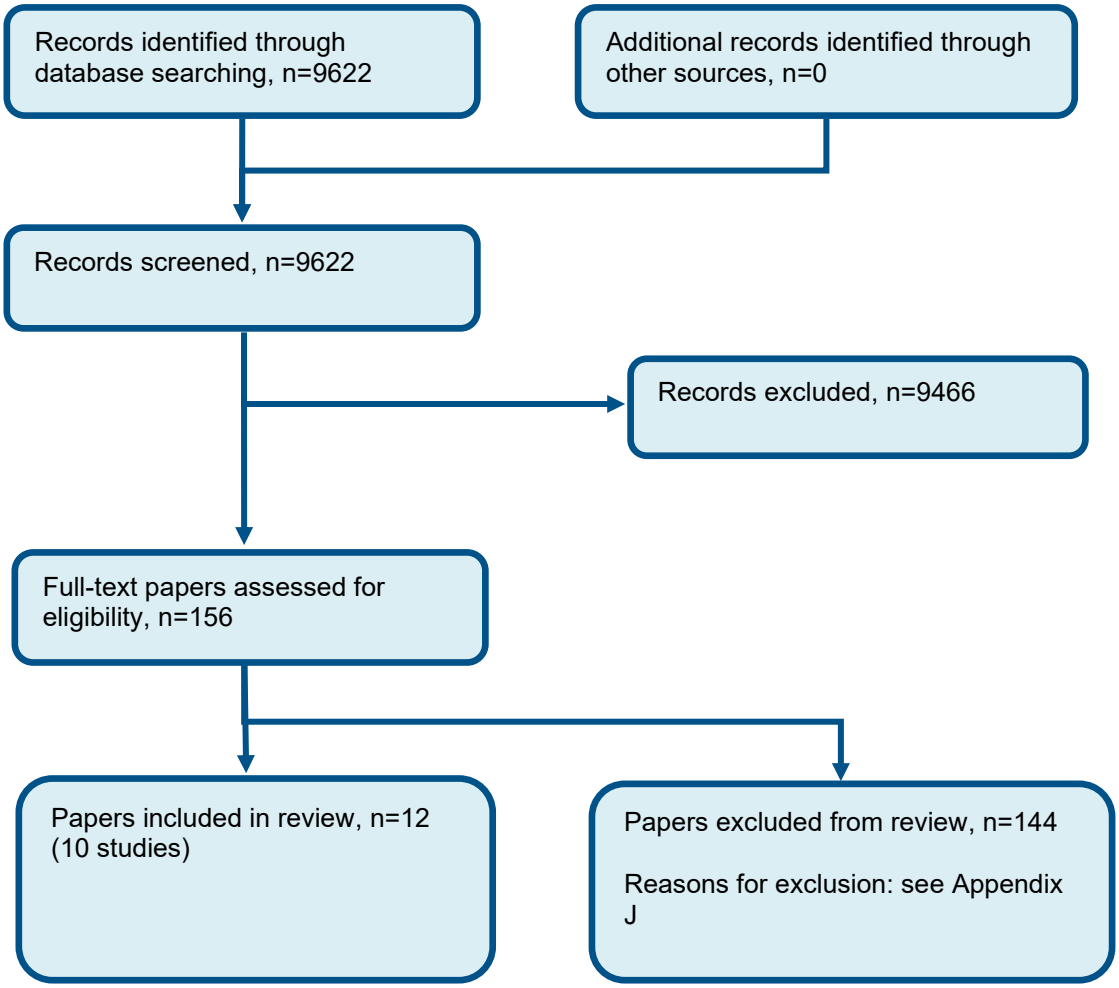
INAHTA search terms

1.	(Anemia, Pernicious)[mh] OR (Vitamin B 12 Deficiency)[mh] OR (pernicious anemia) OR (pernicious anemia) OR (B12) OR (B 12)

Appendix C – Effectiveness evidence study selection

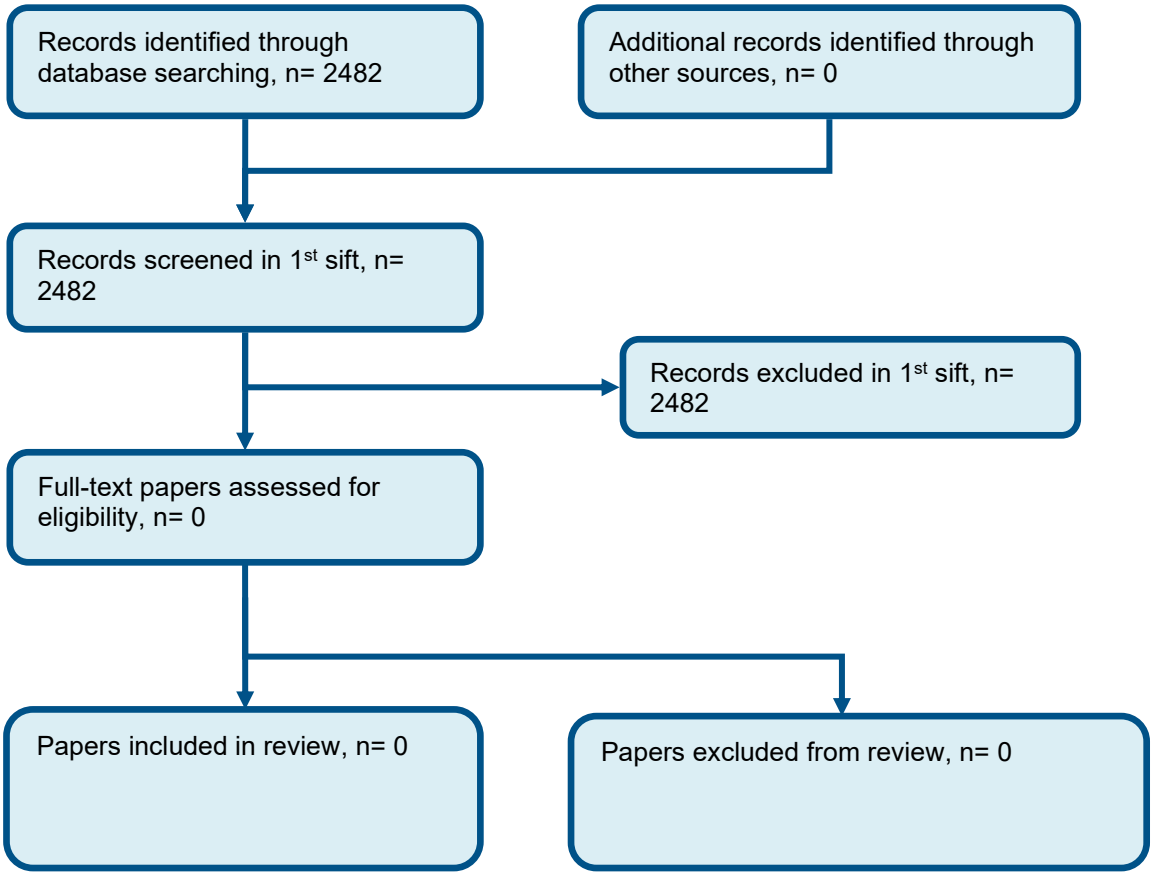
C.1 Diagnostic accuracy

Figure 1: Flow chart of clinical study selection for the review of accuracy of diagnostic tests



C.21 Intervention

Figure 2: Flow chart of clinical study selection for the review of clinical and cost effectiveness of diagnostic tests



Appendix D – Effectiveness evidence

D.1 Diagnostic accuracy

Reference	Bolann 2000[1]
Study type	Diagnostic accuracy observational cohort study
Study methodology	Data source: subjects referred to the Department of Clinical Chemistry at a single hospital by general practitioners for determination of serum cobalamin between June 1994 and November 1996.
Number of patients	n = 196 (187 included in the analysis) Prevalence: 51/187
Patient characteristics	Age, median (range): 59 (17-87) years Pregnancy third trimester: not reported Ethnicity: not reported Gender: mixed. 67.9% female Setting: single hospital Country: Norway Inclusion criteria: 16-90 years of age with serum cobalamin ≤300 pmol/L Exclusion criteria: known haematological or malignant disease, or heart failure Vitamin B12 supplements: not reported
Target condition(s)	Vitamin B12 deficiency
Index test(s) and reference standard	Index test (first line): Serum cobalamin determined by Bio-Rad Quantaphase II Radioassay (June 1994 to March 1995) or Abbott IMx System (from March 1995) (cut off 116 pmol/L) Serum cobalamin determined by Bio-Rad Quantaphase II Radioassay (June 1994 to March 1995) or Abbott IMx System (from March 1995) (cut off 150 pmol/L)

Reference	Bolann 2000[1]
	Plasma total homocysteine determined by published methods (cut off 15 µmol/L) Plasma total homocysteine determined by published methods (cut off 11.3 µmol/L) Reference standard Initial MMA values >0.26 µmol/L (upper reference limit), which subsequently decreased by at least 50% 4 weeks after the start of cobalamin injections (1mg of cyanocobalamin intramuscularly twice weekly for 2.5 weeks). Time interval between reference standard and index test: not reported
Statistical measures	Outcomes: Cobalamin deficiency (initial MMA values >0.26 µmol/L, which subsequently decreased by at least 50% 4 weeks after the start of cobalamin injections) Cobalamin deficiency: serum cobalamin (cut off 116 pmol/L) TP: 37 FP: 35 TN: 101 FN: 14 Sensitivity % 95% CI: 73 (58-84) Specificity% 95% CI: 74 (66-81) PPV % 95% CI: 51 (CI not calculable) NPV % 95% CI: 88 (CI not calculable) Cobalamin deficiency: serum cobalamin (cut off 150 pmol/L) TP: 46 FP: 54 TN: 82 FN: 5 Sensitivity % 95% CI: 90 (79-97) Specificity% 95% CI: 60 (52-69) PPV % 95% CI: 46 (CI not calculable) NPV % 95% CI: 94 (CI not calculable) Cobalamin deficiency: plasma total homocysteine (cut off 15 µmol/L) TP: 37

Reference	Bolann 2000[1]
	FP: 44 TN: 92 FN: 14 Sensitivity % 95% CI: 73 (58-84) Specificity% 95% CI: 68 (59-75) PPV % 95% CI: 46 (CI not calculable) NPV % 95% CI: 87 (CI not calculable) <u>Cobalamin deficiency: plasma total homocysteine (cut off 11.3 µmol/L)</u> TP: 46 FP: 84 TN: 52 FN: 5 Sensitivity % 95% CI: 90 (79-97) Specificity% 95% CI: 38 (30-47) PPV % 95% CI: 35 (CI not calculable) NPV % 95% CI: 91 (CI not calculable)
Source of funding	Not reported
Limitations	Risk of bias (QUADAS 2 – risk of bias): serious due to lack of reporting as to whether index tests and reference standard were conducted and interpreted without knowledge of each other and the time interval between their measurement Indirectness (QUADAS 2 – applicability): very serious due to mixed age strata, lack of reporting regarding use of vitamin B12 supplements and lack of a gold standard test for vitamin B12 deficiency
Comments	Authors compared the 2 methods used for measurement of cobalamin and found no significant bias for serum cobalamin values ≤300 pmol/L TP, FP, TN, FN values and CIs calculated from reported sensitivity/specificity and prevalence data.

1

Reference	Bondu 2020[2]
Study type	Diagnostic accuracy observational cohort study
Study methodology	Data source: patients attending the outpatient department at a Medical College, with a total Vitamin B12 lab request
Number of patients	n = 217 Prevalence: 70/217
Patient characteristics	Age, mean (standard deviation), range: 44.5 (± 13.7), 17-83 years Pregnancy third trimester: not reported

Reference	Bondu 2020[2]
	Ethnicity: not reported Gender: mixed. 54.4% female Setting: single hospital Country: India Inclusion criteria: total Vitamin B12 lab request Exclusion criteria: not reported Vitamin B12 supplements: not reported
Target condition(s)	Vitamin B12 deficiency
Index test(s) and reference standard	Index test (first line): Serum active vitamin B12 (determined by chemiluminescence microparticle automated immunoassay on Siemens ADVIA Centaur xpi system) Total Homocysteine (HCY) (determined by chemiluminescence microparticle automated immunoassay on Siemens ADVIA Centaur xpi system), cut off not reported Reference standard Deficient: serum total vitamin B12 levels below 200 pg/mL Borderline: serum total vitamin B12 levels ranging from 200 to 350 pg/mL Sufficient: serum total vitamin B12 levels >350 pg/mL Time interval between reference standard and index test: not reported
Statistical measures	Outcomes: Total vitamin B12 deficiency (serum total vitamin B12 levels below 200 pg/mL) Total vitamin B12 deficiency and borderline deficiency (serum total vitamin B12 levels ≤350 pg/mL) Total vitamin B12 deficiency: total homocysteine TP: 51 FP: 29

Reference	Bondu 2020[2]
	TN: 118 FN: 19 Sensitivity % 95% CI: 73 (61-83) Specificity% 95% CI: 80 (73-86) PPV % 95% CI: 64 (CI not calculable) NPV % 95% CI: 86 (CI not calculable) <u>Total vitamin B12 deficiency and borderline deficiency: total homocysteine</u> TP: 79 FP: 0 TN: 47 FN: 91 Sensitivity % 95% CI: 46 (39-54) Specificity% 95% CI: 100 (92-100) PPV % 95% CI: 100 (CI not calculable) NPV % 95% CI: 34 (CI not calculable) <u>Total vitamin B12 deficiency: holotranscobalamin</u> TP: 59 FP: 35 TN: 112 FN: 11 Sensitivity % 95% CI: 84 (74-92) Specificity% 95% CI: 76 (68-83) PPV % 95% CI: 63 (CI not calculable) NPV % 95% CI: 91 (CI not calculable) <u>Total vitamin B12 deficiency and borderline deficiency: holotranscobalamin</u> TP: 93 FP: 1 TN: 46 FN: 77 Sensitivity % 95% CI: 55 (47-62) Specificity% 95% CI: 98 (89-100) PPV % 95% CI: 99 (CI not calculable)

Reference	Bondu 2020[2]
	NPV % 95% CI:37 (CI not calculable)
Source of funding	Not reported
Limitations	Risk of bias (QUADAS 2 – risk of bias): very serious due to lack of reporting on patient selection, details of the index tests, whether index tests and reference standard were conducted and interpreted without knowledge of each other and the time interval between their measurement Indirectness (QUADAS 2 – applicability): very serious due to mixed age strata, lack of reporting regarding use of vitamin B12 supplements and lack of a gold standard test for vitamin B12 deficiency
Comments	Sensitivity/specificity calculated from 2x2 tables.

1

Reference	Campos 2020[8]; Campos 2020[3]
Study type	Diagnostic accuracy retrospective observational cohort study
Study methodology	Data source: consecutive routine measurement results from investigations of vitamin B12 status performed between December 2006 and October 2018 in 2 medical laboratories. Clinical samples were from patients referred for either isolated or simultaneous determination of holotranscobalamin (HoloTC), vitamin B12 (B12), methylmalonic acid (MMA), or homocysteine (Hcy). Study 1: Those with a comprehensive assessment of B12 status, including simultaneous measurement of B12, HoloTC, MMA and Hcy were included in the analysis Study 2: Those with a comprehensive assessment of B12 status, including simultaneous measurement of B12, HoloTC, MMA, Hcy and folate were included in the analysis
Number of patients	Study 1: n = 11,833 samples from 9,464 patients Prevalence: 0.49% possible or probable deficiency, 8.2 % (CI 7.7-8.7) subclinical deficiency Study 2: n=3,614 samples from 3,333 patients Prevalence: 8.55% (95% CI 7.68-9.5) inadequate vitamin B12 status
Patient characteristics	Study 1: Age: age range/breakdown not reported, but median (IQR) suggest majority were adults (56 (41-68) years) Pregnancy third trimester: not reported Ethnicity: not reported Gender: mixed. 58.8% female Setting: 2 medical laboratories Country: Switzerland and Liechtenstein Inclusion criteria: comprehensive assessment of B12 status, including simultaneous measurement of B12, HoloTC, MMA and Hcy Exclusion criteria: not reported

Reference	Campos 2020[8]; Campos 2020[3]
	Vitamin B12 supplements: not reported Study 2: Age: age range/breakdown not reported, but median (IQR) suggest majority were adults (53 (40-64) years) Pregnancy third trimester: not reported Ethnicity: not reported Gender: mixed. 54.9% female Setting: 2 medical laboratories Country: Switzerland and Liechtenstein Inclusion criteria: comprehensive assessment of B12 status, including simultaneous measurement of B12, HoloTC, MMA, Hcy and folate Exclusion criteria: not reported Vitamin B12 supplements: not reported
Target condition(s)	Vitamin B12 deficiency
Index test(s) and reference standard	Index test (first line): Study 1: HoloTC (assayed with commercially available immunoassay); optimum cut off with least misclassification <27 pmol/L for possible/probable deficiency, <45 pmol/L for subclinical deficiency HoloTC (assayed with commercially available immunoassay); cut off at 99% sensitivity <56.5 pmol/L for possible/probable deficiency, <73 pmol/L for subclinical deficiency HoloTC (assayed with commercially available immunoassay); cut off at 99% specificity <19 pmol/L for possible/probable deficiency, <25 pmol/L for subclinical deficiency B12 (assayed with commercially available immunoassay); optimum cut off with least misclassification <167 pmol/L for possible/probable deficiency, <229 pmol/L for subclinical deficiency B12 (assayed with commercially available immunoassay); cut off at 99% sensitivity <320 pmol/L for possible/probable deficiency, <351 pmol/L for subclinical deficiency B12 (assayed with commercially available immunoassay); cut off at 99% specificity <115 pmol/L for possible/probable deficiency, <142 pmol/L for subclinical deficiency MMA (measured by a LC/MS-MS system); optimum cut off with least misclassification >466 nmol/L for possible/probable deficiency, >245 nmol/L for subclinical deficiency MMA (measured by a LC/MS-MS system); cut off at 99% sensitivity >158 nmol/L for possible/probable deficiency, >152 nmol/L for subclinical deficiency

Reference	Campos 2020[8]; Campos 2020[3]
	MMA (measured by a LC/MS-MS system); cut off at 99% specificity >723 nmol/L for possible/probable deficiency, >480 nmol/L for subclinical deficiency Hcy (assayed with commercially available immunoassay); optimum cut off with least misclassification >16,4 µmol/L for possible/probable deficiency, >15 µmol/L for subclinical deficiency Hcy (assayed with commercially available immunoassay); cut off at 99% sensitivity >6.2 µmol/L for possible/probable deficiency, >8 µmol/L for subclinical deficiency Hcy (assayed with commercially available immunoassay); cut off at 99% specificity >34 µmol/L for possible/probable deficiency, >29 µmol/L for subclinical deficiency Study 2: Harrington's algorithm – 2-step diagnostic algorithm that comprises measurement of HoloTC as a first step, and if the result is 25-70 pmol/L, a subsequent measurement of MMA (as a second step) is performed. If MMA is <280 nmol/L (or <360 nmol/L in patients aged >65 years), vitamin B12 sufficiency can be assumed, whereas MMA ≥280 nmol/L (≥360 nmol/L in patients ≥65 years), vitamin B12 deficiency can be postulated. $2cB12_{HoloTC/MMA}$ $2cB12_{B12/MMA}$ $2cB12_{B12/Hcy}$ $2cB12_{HoloTC/B12}$ $2cB12_{HoloTC/Hcy}$ $2cB12_{MMA/Hcy}$ $3cB12_{HoloTC/B12/MMA}$ $3cB12_{MMA/HoloTC/Hcy}$ $3cB12_{HoloTC/B12/Hcy}$ $3cB12_{MMA/B12/Hcy}$ Reference standard

Reference	Campos 2020[8]; Campos 2020[3]
	4cB12 Integrates the direct markers (HoloTC and B12) in pmol/L and metabolic markers (MMA and Hcy) in µmol/L of B12 deficiency and age based on models obtained from large empirical investigations. 4cB12 can be obtained according to the following equation: $4cB12 = \log_{10} \frac{HoloTc \times B12}{MMA \times Hcy} - \frac{3:79}{1 + (age/230)^{2.6'}}$ 4cB12 ≤−0:5 was defined as an indicator of low B12, with at least potential subclinical manifestations of B12 deficiency. A value <−1:5 indicates possible and probable B12 deficiency. Time interval between reference standard and index test: tests conducted using the same sample.
Statistical measures	Outcomes: Study 1: Possible or probable B12 deficiency (4cB12 ≤ -1.5) Subclinical B12 deficiency (4cB12 ≤ -0.5 and >-1.5) <u>Possible or probable B12 deficiency: HoloTC; cut off <27 pmol/L</u> TP: 54 FP: 471 TN: 11304 FN: 4 Sensitivity % 95% CI: 93.1 (83-98) Specificity% 95% CI: 96 (96-96) PPV % 95% CI: 10 (CI not calculable) NPV % 95% CI: 100 (CI not calculable) <u>Possible or probable B12 deficiency: HoloTC; cut off <56.5 pmol/L</u> TP: 57 FP: 4710 TN: 7065 FN: 1 Sensitivity % 95% CI: 99 (91-100) Specificity% 95% CI: 60 (59-61) PPV % 95% CI: 1 (CI not calculable) NPV % 95% CI: 100 (CI not calculable)

Reference	Campos 2020[8]; Campos 2020[3]
	Possible or probable B12 deficiency: HoloTC; cut off <19 pmol/L TP: 45 FP: 118 TN: 11657 FN: 13 Sensitivity % 95% CI: 77.6 (65-87) Specificity% 95% CI: 99 (99-99) PPV % 95% CI: 28 (CI not calculable) NPV % 95% CI: 100 (CI not calculable) Possible or probable B12 deficiency: B12; cut off <167 pmol/L TP: 55 FP: 907 TN: 10868 FN: 3 Sensitivity % 95% CI: 94.8 (86-99) Specificity% 95% CI: 92.3 (92-93) PPV % 95% CI: 6 (CI not calculable) NPV % 95% CI: 100 (CI not calculable) Possible or probable B12 deficiency: B12; cut off <320 pmol/L TP: 57 FP: 6877 TN: 4898 FN: 1 Sensitivity % 95% CI: 99 (91-100) Specificity% 95% CI: 41.6 (41-42) PPV % 95% CI: 1 (CI not calculable) NPV % 95% CI: 100 (CI not calculable) Possible or probable B12 deficiency: B12; cut off <115 pmol/L TP: 33 FP: 118 TN: 11657

Reference	Campos 2020[8]; Campos 2020[3]
	FN: 25 Sensitivity % 95% CI: 56.9 (43-70) Specificity% 95% CI: 99 (99-99) PPV % 95% CI: 22 (CI not calculable) NPV % 95% CI: 100 (CI not calculable) <u>Possible or probable B12 deficiency: MMA; cut off >466 nmol/L</u> TP: 55 FP: 424 TN: 11351 FN: 3 Sensitivity % 95% CI: 94.8 (86-99) Specificity% 95% CI: 96.4 (96-97) PPV % 95% CI: 11 (CI not calculable) NPV % 95% CI: 100 (CI not calculable) <u>Possible or probable B12 deficiency: MMA; cut off >158 nmol/L</u> TP: 57 FP: 7171 TN: 4604 FN: 1 Sensitivity % 95% CI: 99 (91-100) Specificity% 95% CI: 39.1 (38-40) PPV % 95% CI: 1 (CI not calculable) NPV % 95% CI: 100 (CI not calculable) <u>Possible or probable B12 deficiency: MMA; cut off >723 nmol/L</u> TP: 42 FP: 118 TN: 11657 FN: 16 Sensitivity % 95% CI: 72.4 (59-83) Specificity% 95% CI: 99 (99-99) PPV % 95% CI: 26 (CI not calculable) NPV % 95% CI: 100 (CI not calculable)

Reference	Campos 2020[8]; Campos 2020[3]
	Possible or probable B12 deficiency: Hcy; cut off >16,4 µmol/L TP: 51 FP: 2249 TN: 9526 FN: 7 Sensitivity % 95% CI: 87.9 (77-95) Specificity% 95% CI: 80.9 (80-82) PPV % 95% CI: 2 (CI not calculable) NPV % 95% CI: 100 (CI not calculable) Possible or probable B12 deficiency: Hcy; cut off >6.2 µmol/L TP: 57 FP: 11422 TN: 353 FN: 1 Sensitivity % 95% CI: 99 (91-100) Specificity% 95% CI: 3 (3-3) PPV % 95% CI: 1 (CI not calculable) NPV % 95% CI: 100 (CI not calculable) Possible or probable B12 deficiency: Hcy; cut off >34 µmol/L TP: 19 FP: 118 TN: 11657 FN: 39 Sensitivity % 95% CI: 32.8 (21-46) Specificity% 95% CI: 99 (99-99) PPV % 95% CI: 14 (CI not calculable) NPV % 95% CI: 100 (CI not calculable) Subclinical B12 deficiency: HoloTC; cut off <45 pmol/L TP: 831 FP: 2042 TN: 8821

Reference	Campos 2020[8]; Campos 2020[3]
	FN: 139 Sensitivity % 95% CI: 85.7 (83-88) Specificity% 95% CI: 81.2 (80-82) PPV % 95% CI: 29 (CI not calculable) NPV % 95% CI: 98 (CI not calculable) <u>Subclinical B12 deficiency: HoloTC; cut off <73 pmol/L</u> TP: 960 FP: 6072 TN: 4791 FN: 10 Sensitivity % 95% CI: 99 (98-100) Specificity% 95% CI: 44.1 (43-45) PPV % 95% CI: 14 (CI not calculable) NPV % 95% CI: 100 (CI not calculable) <u>Subclinical B12 deficiency: HoloTC; cut off <25 pmol/L</u> TP: 267 FP: 109 TN: 10754 FN: 703 Sensitivity % 95% CI: 27.5 (25-30) Specificity% 95% CI: 99 (99-99) PPV % 95% CI: 71 (CI not calculable) NPV % 95% CI: 94 (CI not calculable) <u>Subclinical B12 deficiency: B12; cut off <229 pmol/L</u> TP: 835 FP: 2422 TN: 8441 FN: 135 Sensitivity % 95% CI: 86.1 (84-88) Specificity% 95% CI: 77.7 (77-78) PPV % 95% CI: 26 (CI not calculable) NPV % 95% CI: 98 (CI not calculable)

Reference	Campos 2020[8]; Campos 2020[3]
	Subclinical B12 deficiency: B12; cut off <351 pmol/L TP: 960 FP: 6876 TN: 3987 FN: 10 Sensitivity % 95% CI: 99 (98-100) Specificity% 95% CI: 36.7 (36-38) PPV % 95% CI: 12 (CI not calculable) NPV % 95% CI: 100 (CI not calculable) Subclinical B12 deficiency: B12; cut off <142 pmol/L TP: 273 FP: 109 TN: 10754 FN: 697 Sensitivity % 95% CI: 28.2 (25-31) Specificity% 95% CI: 99 (99-99) PPV % 95% CI: 72 (CI not calculable) NPV % 95% CI: 94 (CI not calculable) Subclinical B12 deficiency: MMA; cut off >245 nmol/L TP: 793 FP: 1803 TN: 9060 FN: 177 Sensitivity % 95% CI: 81.8 (79-84) Specificity% 95% CI: 83.4 (83-84) PPV % 95% CI: 31 (CI not calculable) NPV % 95% CI: 98 (CI not calculable) Subclinical B12 deficiency: MMA; cut off >152 nmol/L TP: 960 FP: 6757 TN: 4106

Reference	Campos 2020[8]; Campos 2020[3]
	FN: 10 Sensitivity % 95% CI: 99 (98-100) Specificity% 95% CI: 37.8 (37-39) PPV % 95% CI: 12 (CI not calculable) NPV % 95% CI: 100 (CI not calculable) <u>Subclinical B12 deficiency: MMA; cut off >480 nmol/L</u> TP: 284 FP: 109 TN: 10754 FN: 686 Sensitivity % 95% CI: 29.3 (26-32) Specificity% 95% CI: 99 (99-99) PPV % 95% CI: 72 (CI not calculable) NPV % 95% CI: 94 (CI not calculable) <u>Subclinical B12 deficiency: Hcy; cut off >15 µmol/L</u> TP: 657 FP: 2531 TN: 8332 FN: 313 Sensitivity % 95% CI: 67.7 (65-71) Specificity% 95% CI: 76.7 (76-77) PPV % 95% CI: 21 (CI not calculable) NPV % 95% CI: 96 (CI not calculable) <u>Subclinical B12 deficiency: Hcy; cut off >8 µmol/L</u> TP: 960 FP: 9494 TN: 1369 FN: 10 Sensitivity % 95% CI: 99 (98-100) Specificity% 95% CI: 12.6 (12-13) PPV % 95% CI: 9 (CI not calculable) NPV % 95% CI: 99 (CI not calculable)

Reference	Campos 2020[8]; Campos 2020[3]
	Subclinical B12 deficiency: Hcy; cut off >29 µmol/L TP: 111 FP: 109 TN: 10754 FN: 859 Sensitivity % 95% CI: 11.5 (10-14) Specificity% 95% CI: 99 (99-99) PPV % 95% CI: 51 (CI not calculable) NPV % 95% CI: 93 (CI not calculable) Study 2: Inadequate vitamin B12 status (4cB12 <-0.5) Inadequate vitamin B12 status: Harrington's algorithm TP: 256 FP: 231 TN: 3074 FN: 53 Sensitivity % 95% CI: 83 (78-87) Specificity% 95% CI: 93 (92-94) PPV % 95% CI: 53 (CI not calculable) NPV % 95% CI: 98 (CI not calculable) PLR: 12.1 NLR: 0.18 Inadequate vitamin B12 status: 2cB12$_{HoloTC/MMA}$ TP: 235 FP: 66 TN: 3239 FN: 74 Sensitivity % 95% CI: 76 (71-81) Specificity% 95% CI: 98 (97-98) PPV % 95% CI: 78 (CI not calculable) NPV % 95% CI: 98 (CI not calculable)

Reference	Campos 2020[8]; Campos 2020[3]
	PLR: 42.6 NLR: 0.24 <u>Inadequate vitamin B12 status: 2cB12$_{B12/MMA}$</u> TP: 173 FP: 33 TN: 3272 FN: 136 Sensitivity % 95% CI: 56 (50-62) Specificity% 95% CI: 99 (99-99) PPV % 95% CI: 84 (CI not calculable) NPV % 95% CI: 96 (CI not calculable) PLR: 66.1 NLR: 0.44 <u>Inadequate vitamin B12 status: 2cB12$_{B12/Hcy}$</u> TP: 210 FP: 165 TN: 3140 FN: 99 Sensitivity % 95% CI: 68 (62-73) Specificity% 95% CI: 95 (94-96) PPV % 95% CI: 56 (CI not calculable) NPV % 95% CI: 97 (CI not calculable) PLR: 13.3 NLR: 0.34 <u>Inadequate vitamin B12 status: 2cB12$_{HoloTC/B12}$</u> TP: 244 FP: 132 TN: 3173 FN: 65 Sensitivity % 95% CI: 79 (74-83) Specificity% 95% CI: 96 (95-97) PPV % 95% CI: 65 (CI not calculable)

Reference	Campos 2020[8]; Campos 2020[3]
	NPV % 95% CI: 98 (CI not calculable) PLR: 19.1 NLR: 0.22 Inadequate vitamin B12 status: 2cB12$_{HoloTC/Hcy}$ TP: 284 FP: 331 TN: 2974 FN: 25 Sensitivity % 95% CI: 92 (88-95) Specificity% 95% CI: 90 (89-91) PPV % 95% CI: 46 (CI not calculable) NPV % 95% CI: 99 (CI not calculable) PLR: 9.3 NLR: 0.09 Inadequate vitamin B12 status: 2cB12$_{MMA/Hcy}$ TP: 207 FP: 165 TN: 3140 FN: 102 Sensitivity % 95% CI: 67 (61-72) Specificity% 95% CI: 95 (94-96) PPV % 95% CI: 56 (CI not calculable) NPV % 95% CI: 97 (CI not calculable) PLR: 12.9 NLR: 0.34 Inadequate vitamin B12 status: 3cB12$_{HoloTC/B12/MMA}$ TP: 241 FP: 33 TN: 3272 FN: 68 Sensitivity % 95% CI: 78 (73-82) Specificity% 95% CI: 99 (99-99)

Reference	Campos 2020[8]; Campos 2020[3]
	PPV % 95% CI: 88 (CI not calculable) NPV % 95% CI: 98 (CI not calculable) PLR: 82.8 NLR: 0.23 Inadequate vitamin B12 status: 3cB12$_{MMA/HoloTC/Hcy}$ TP: 269 FP: 66 TN: 3239 FN: 40 Sensitivity % 95% CI: 87 (83-91) Specificity% 95% CI: 98 (97-98) PPV % 95% CI: 80 (CI not calculable) NPV % 95% CI: 99 (CI not calculable) PLR: 40.7 NLR: 0.13 Inadequate vitamin B12 status: 3cB12$_{HoloTC/B12/Hcy}$ TP: 269 FP: 132 TN: 3173 FN: 40 Sensitivity % 95% CI: 87 (83-91) Specificity% 95% CI: 96 (95-97) PPV % 95% CI: 67 (CI not calculable) NPV % 95% CI: 99 (CI not calculable) PLR: 20.2 NLR: 0.13 Inadequate vitamin B12 status: 3cB12$_{MMA/B12/Hcy}$ TP: 207 FP: 33 TN: 3272 FN: 102 Sensitivity % 95% CI: 67 (61-72)

Reference	Campos 2020[8]; Campos 2020[3]
	Specificity% 95% CI: 99 (99-99) PPV % 95% CI: 86 (CI not calculable) NPV % 95% CI: 97 (CI not calculable) PLR: 61.8 NLR: 0.33
Source of funding	Not reported
Limitations	Risk of bias (QUADAS 2 – risk of bias): serious due to patient selection bias (analysis based on samples rather than patients) Indirectness (QUADAS 2 – applicability): very serious due to lack of reporting regarding use of vitamin B12 supplements and lack of a gold standard test for vitamin B12 deficiency
Comments	Study 1: Separate analyses reported for males and females and age < 50 and age ≥ 50 years, but AUCs only. No sensitivity/specificity data. AUC gives an overall measure of accuracy of the test across a range of thresholds. Study 1 and 2: TP, FP, TN, FN and CIs calculated from reported sensitivity/specificity and prevalence data.

1

2

Reference	Goringe 2006[4]
Study type	Diagnostic accuracy observational cohort study
Study methodology	Data source: patients recruited from those referred to the Haematology laboratory of an NHS trust for B12 estimation between June 2002 and July 2003. Samples received from general practitioners for vitamin B12 assay
Number of patients	n = 49 (n=27 with low Hb concentration at first hospital visit and/or macrocytosis (pre-treatment MCV of 97 fL or more) included in the analysis) Prevalence: not reported
Patient characteristics	Age: mixed. <75 years (no further information reported) Pregnancy third trimester: not reported Ethnicity: not reported Gender: mixed. 71.4% female Setting: Haematology laboratory of an NHS trust Country: Wales Inclusion criteria: serum B12 <170 ng/L

Reference	Goringe 2006[4]
	Exclusion criteria: abnormal liver function tests, hypothyroidism, alcohol abuse, folate deficiency, or renal failure Vitamin B12 supplements: not reported
Target condition(s)	Vitamin B12 deficiency
Index test(s) and reference standard	Index test (second line): Methylmalonic acid (in house GCMS), cut off >0.47 µmol/L Holotranscobalamin (Axis Shield), cut off <38 pmol/L Homocysteine (fluorescence polarization immunoassay, Abbot IMX), cut off >15 µmol/L Reference standard Response to treatment with intramuscular B12 injections (1mg per week for 4 weeks), defined as an increase in Hb concentration of 1 g/dL or more and/or a decrease in MCV of 3 fl or more. Patients reassessed at 3 months. Time interval between reference standard and index test: 3 months
Statistical measures	Outcomes: Response to treatment (increase in Hb concentration of 1 g/dL or more and/or a decrease in MCV of 3 fl or more) Response to treatment: MMA (cut off >0.47 µmol/L) TP: 11 FP: 4 TN: 8 FN: 4 Sensitivity % 95% CI: 73 (45-92) Specificity% 95% CI: 67 (35-90) PPV % 95% CI: 73 (CI not calculable) NPV % 95% CI: 67 (CI not calculable) Response to treatment: HoloTC (cut off <38 pmol/L) TP: 15 FP: 12

Reference	Goringe 2006[4]
	TN: 0 FN: 0 Sensitivity % 95% CI: 100 (78-100) Specificity% 95% CI: 0 (0-26) PPV % 95% CI: 56 (CI not calculable) NPV % 95% CI: not calculable <u>Response to treatment: tHCY (cut off >15 µmol/L)</u> TP: 15 FP: 7 TN: 5 FN: 0 Sensitivity % 95% CI: 100 (78-100) Specificity% 95% CI: 42 (15-72) PPV % 95% CI: 68 (CI not calculable) NPV % 95% CI: 100 (CI not calculable)
Source of funding	Not reported
Limitations	Risk of bias (QUADAS 2 – risk of bias): serious due to lack of reporting as to whether index tests and reference standard were conducted and interpreted without knowledge of each other and the 3-month time interval between their measurement Indirectness (QUADAS 2 – applicability): very serious due to unclear reporting on age, lack of reporting regarding use of vitamin B12 supplements, only those who were anaemic or macrocytic being included in the analysis and lack of a gold standard test for vitamin B12 deficiency
Comments	Sensitivity/specificity calculated from 2x2 tables. Study also reported results for low initial serum B12, but not extracted as only those with low serum B12 were included in the analysis. Those (n=22) with normal initial Hb concentration and MCV <97 g/dL are not included in the analysis. Symptomatic improvement did not correlate with hematologic response to treatment.

1

Reference	Heil 2012[5]
Study type	Diagnostic accuracy observational cohort study
Study methodology	Data source: patient samples collected between November 2006 and July 2007 from clinical chemistry laboratories. Each centre aimed to collect samples of 250 patients of whom vitamin B12 was requested. Each centre was asked to select 20 patients with a vitamin B12 concentration below 100 pmol/L, 80 patients with vitamin B12 between 100 and 200 pmol/L, 100 patients with vitamin B12 between 200 and 300 pmol/L and 50 patients with vitamin B12 above 300 pmol/L.
Number of patients	n = 360 Prevalence: 13%

Reference	Heil 2012[5]
Patient characteristics	Age, mean (range): mixed. 59 (19-100) Pregnancy third trimester: not reported Ethnicity: not known Gender: mixed. 62.2% female Setting: 5 hospitals Country: Netherlands Inclusion criteria: ≥18 years, normal renal function (estimated glomerular filtration rate [eGFR] ≥60 mL/min/1.73 m^2) and availability of vitamin B12, holoTC and MMA measurements in serum Exclusion criteria: not reported Vitamin B12 supplements: not reported
Target condition(s)	Vitamin B12 deficiency
Index test(s) and reference standard	Index test (first line): Serum vitamin B12 (AxSYM; Abbott,), cut off <145 pmol/L (reference range 145– 637 pmol/L (95% reference range determined in 100 healthy blood bank donors)) Serum vitamin B12 (AxSYM; Abbott,), cut off <180 pmol/L for optimal sensitivity derived from ROC plot (reference range 145– 637 pmol/L (95% reference range determined in 100 healthy blood bank donors)) HoloTC (AxSYM analyser (Abbott)), cut off <21 pmol/L (reference range 21 –117 pmol/L (95% reference range determined in 100 healthy blood bank donors)) HoloTC (AxSYM analyser (Abbott)), cut off for optimal sensitivity derived from ROC plot <32 pmol/L (reference range 21 –117 pmol/L (95% reference range determined in 100 healthy blood bank donors)) Reference standard Serum MMA measured by tandem mass spectrometry (LC-MS/MS), reference range 0.09 –0.45 µmol/L (95% reference range determined in 100 healthy blood bank donors). Three predefined cut-off values of MMA were applied to define metabolic vitamin B12 deficiency: MMA >0.32 µmol/L (i.e. 90th percentile); serum MMA >0.45 µmol/L (i.e. 97.5th percentile); and MMA >0.77 µmol/L (i.e. 99th percentile).

Reference	Heil 2012[5]
	Time interval between reference standard and index test: same sample used for reference and index tests.
Statistical measures	Outcomes: Metabolic vitamin B12 deficiency (MMA >0.45 µmol/L) <u>Metabolic vitamin B12 deficiency: serum vitamin B12 (cut off <145 pmol/L)</u> TP: 25 FP: 60 TN: 253 FN: 22 Sensitivity % 95% CI: 53 (38-68) Specificity% 95% CI: 81 (76-85) PPV % 95% CI: 29 (CI not calculable) NPV % 95% CI: 92 (CI not calculable) <u>Metabolic vitamin B12 deficiency: serum vitamin B12 (cut off <180 pmol/L)</u> TP: 30 FP: 113 TN: 200 FN: 17 Sensitivity % 95% CI: 64 (49-77) Specificity% 95% CI: 64 (58-69) PPV % 95% CI: 21 (CI not calculable) NPV % 95% CI: 92 (CI not calculable) <u>Metabolic vitamin B12 deficiency: HoloTC (cut off <21 pmol/L)</u> TP: 30 FP: 38 TN: 275 FN: 17 Sensitivity % 95% CI: 64 (49-77) Specificity% 95% CI: 88 (84-91) PPV % 95% CI: 44 (CI not calculable) NPV % 95% CI: 94 (CI not calculable)

Reference	Heil 2012[5]
	Metabolic vitamin B12 deficiency: HoloTC (cut off <32 pmol/L) TP: 39 FP: 125 TN: 188 FN: 8 Sensitivity % 95% CI: 83 (69-92) Specificity% 95% CI: 60 (54-66) PPV % 95% CI: 24 (CI not calculable) NPV % 95% CI: 96 (CI not calculable)
Source of funding	Assays for holoTC and vitamin B12 provided by Abbott.
Limitations	Risk of bias (QUADAS 2 – risk of bias): serious due to unclear method of patient selection and lack of reporting as to whether index tests and reference standard were conducted and interpreted without knowledge of each other Indirectness (QUADAS 2 – applicability): very serious due to mixed age strata, lack of reporting regarding use of vitamin B12 supplements and lack of a gold standard test for vitamin B12 deficiency
Comments	Authors state that they evaluated diagnostic accuracy of different combinations of vitamin B12 and holoTC and compared this with a single test result, but no useable data are reported. TP, FP, TN, FN and CIs calculated from reported sensitivity/specificity and prevalence data.

1

Reference	Herrmann 2013[6]
Study type	Diagnostic accuracy observational cohort study
Study methodology	Data source: samples referred to a single laboratory for total vitamin B12 measurement. Samples were anonymous and no clinical information available.
Number of patients	n = 1359 Prevalence: 445/1359 (32.75%), 192/1034 (18.57%) in those with serum creatinine ≤ 97.2 µM
Patient characteristics	Age, median (10-90th percentiles): mixed. Reported by percentile of holoTC, ranging from 51 (25-76) years to 71 (47-87) years Pregnancy third trimester: not reported Ethnicity: not reported Gender: not reported Setting: single laboratory Country: Germany

Reference	Herrmann 2013[6]
	Inclusion criteria: samples referred for total vitamin B12 measurement Exclusion criteria: not reported Vitamin B12 supplements: not reported
Target condition(s)	Vitamin B12 deficiency
Index test(s) and reference standard	Index test (first line): Serum total vitamin B12 determined by a chemiluminescence immunoassay (ADVIA Centaur System, Bayer, Germany), cut off 227 pM Holotranscobalamin (HoloTC) measured using a specific monoclonal antibody against holoTC (AxSYM, Abbott, Germany), cut off 35 pM based on literature Holotranscobalamin (HoloTC) measured using a specific monoclonal antibody against holoTC (AxSYM, Abbott, Germany), cut off 22 pM dependent on 90% sensitivity Holotranscobalamin (HoloTC) measured using a specific monoclonal antibody against holoTC (AxSYM, Abbott, Germany), cut off 76 pM dependent on 90% specificity Reference standard Methylmalonic acid (MMA) measured using gas chromatography-mass spectrometry (Agilent Technologies), cut off 300 nM Time interval between reference standard and index test: same sample used for reference and index tests.
Statistical measures	Outcomes: Vitamin B12 deficiency (MMA > 300nM) Vitamin B12 deficiency (MMA > 300nM): Serum total vitamin B12 (cut off 227 pM) TP: 320 FP: 539 TN: 375 FN: 125 Sensitivity % 95% CI: 72 (67-76) Specificity% 95% CI: 41 (38-44) PPV % 95% CI: 37 (CI not calculable) NPV % 95% CI: 75 (CI not calculable)

Reference	Herrmann 2013[6]
	Vitamin B12 deficiency (MMA > 300nM): HoloTC (cut off < 35 pM) TP: 320 FP: 420 TN: 494 FN: 125 Sensitivity % 95% CI: 72 (67-76) Specificity% 95% CI: 54 (51-57) PPV % 95% CI: 43 (CI not calculable) NPV % 95% CI: 80 (CI not calculable) Vitamin B12 deficiency (MMA > 300nM): HoloTC (cut off < 22 pM), (using data from 1034 samples with serum creatinine ≤ 97.2 µM) TP: 173 FP: 615 TN: 227 FN: 19 Sensitivity % 95% CI: 90 (85-94) Specificity% 95% CI: 27 (24-30) PPV % 95% CI: 22 (CI not calculable) NPV % 95% CI: 92 (CI not calculable) Vitamin B12 deficiency (MMA > 300nM): HoloTC (cut off < 76 pM), (using data from 1034 samples with serum creatinine ≤ 97.2 µM) TP: 40 FP: 84 TN: 758 FN: 152 Sensitivity % 95% CI: 21 (15-27) Specificity% 95% CI: 90 (88-92) PPV % 95% CI: 32 (CI not calculable) NPV % 95% CI: 83 (CI not calculable)
Source of funding	Not reported
Limitations	Risk of bias (QUADAS 2 – risk of bias): serious due to lack of reporting on methods of patient selection, patient characteristics and whether index tests and reference standard were conducted and interpreted without knowledge of each other Indirectness (QUADAS 2 – applicability): very serious due to mixed age strata, lack of reporting regarding use of vitamin B12 supplements and lack of a gold standard test for vitamin B12 deficiency

Reference	**Herrmann 2013[6]**
Comments	Study reports a diagnostic algorithm using HoloTC as a first line marker and MMA as a second line marker, but no diagnostic accuracy measures are reported. TP, FP, TN, FN and CIs calculated from reported sensitivity/specificity and prevalence data.

1

Reference	**Holleland 1999[7]**
Study type	Diagnostic accuracy retrospective observational cohort study
Study methodology	Data source: Patients with s-cobalamin concentrations <300 pmol/L from a total of 76,840 cobalamin analyses performed at a single laboratory in 1993. Approximately 75 patients in each of the following scobalamin concentrations were included: 0–139, 140–169, 170–189, 190–219, and 220–299 pmol/L. Only one patient per general practitioner was included.
Number of patients	n = 376 (n=224 included in the analysis) Prevalence: 2.96%
Patient characteristics	Age, median (range): mixed. Medians reported by serum cobalamin interval ranging from 59 to 69 (18-90) years Pregnancy third trimester: not reported Ethnicity: not reported Gender: mixed. Female/male ratio reported by serum cobalamin interval 1.3, 1.6, 2.9, 1.5, 1.9 Setting: single laboratory Country: Norway Inclusion criteria: s-cobalamin concentrations <300 pmol/L Exclusion criteria: doctors were not general practitioners, questionnaires were incompletely filled out, s-cobalamin ordered as a confirmation test of either an earlier s-cobalamin determination or as therapy control in patients on cobalamin supplementation, not enough serum left for measurement Vitamin B12 supplements: not reported but s-cobalamin ordered as therapy control in patients on cobalamin supplementation was excluded
Target condition(s)	Vitamin B12 deficiency
Index test(s) and reference standard	<u>Index test (first line):</u> Serum cobalamin measured by RIA (Diagnostic Product Corp, cut off ≤170 pmol/L (reference range 170–700 pmol/L)

Reference	**Holleland 1999[7]**
	Reference standard Methylmalonic acid (MMA) measured by capillary electrophoresis, cut-off for diagnosing functional cobalamin deficiency was set to 0.376 µmol/L Time interval between reference standard and index test: same sample used for reference and index tests.
Statistical measures	Outcomes: Functional cobalamin deficiency (MMA >0.376 µmol/L) Functional cobalamin deficiency (MMA >0.376 µmol/L): Serum cobalamin (cut off ≤170 pmol/L) TP: 3 FP: 4 TN: 213 FN: 4 Sensitivity % 95% CI: 40 (22-58) Specificity% 95% CI: 98 (97.6-98.3) PPV % 95% CI: 38 (CI not calculable) NPV % 95% CI: 98 (CI not calculable)
Source of funding	Not reported
Limitations	Risk of bias (QUADAS 2 – risk of bias): serious due to methods of patient selection and lack of reporting regarding whether index tests and reference standard were conducted and interpreted without knowledge of each other Indirectness (QUADAS 2 – applicability): very serious due to mixed age strata and lack of a gold standard test for vitamin B12 deficiency
Comments	Sensitivity and specificity analysis was corrected for the frequency distribution of s-cobalamin values. Study also reports sensitivity and specificity data for clinical decision to supplement. Study also reports a model of the diagnostic benefit of MMA, but no extractable diagnostic accuracy measures are reported. TP, FP, TN, FN calculated from reported sensitivity/specificity and prevalence data.

1

Reference	**Matchar 1987[10]; Matchar 1994[11]**
Study type	Diagnostic accuracy prospective observational cohort study
Study methodology	Data source: all patients having serum vitamin B12 levels measured in 1984 at a veterans administration single medical centre

Reference	**Matchar 1987[10]; Matchar 1994[11]**
Number of patients	n = 136 (n=96 with evaluable MMA results, complete follow up and clinical diagnosis included in the MMA analysis; n=134 with complete follow up and clinical diagnosis included in the vitamin B12 analysis) Prevalence: 7.4% (MMA analysis), 11.94 (B12 analysis)
Patient characteristics	Age, mean (standard deviation): 61.6 (11.7) years Pregnancy third trimester: not reported Ethnicity: 69% white Gender: 2% female Setting: single veterans administration medical centre Country: USA Inclusion criteria: patients with low serum B12 levels (<180 pg/mL) and a random sample of patients with normal serum B12 assay results matched by assay date Exclusion criteria: living >1 hour from the hospital and could not reliably keep follow up appointments, died before first evaluation Vitamin B12 supplements: not reported Other comments: indications for serum B12 assay request macrocytosis without anaemia (21%), anaemia without macrocytosis (20%), neuropathy (15%), dementia (15%), macrocytosis with anaemia (13%), miscellaneous (16%)
Target condition(s)	Vitamin B12 deficiency
Index test(s) and reference standard	Index test (first line): Urinary methylmalonic acid measured from a spot urine sample using an isotope dilution assay by combined gas chromatography-mass spectrometry. Normal urinary MMA defined as ≤5 µg /mg creatinine Serum vitamin B12 assay using the SimulTRAC-S kit (Becton-Dickinson Immunodiagnostics, New York) from January to June 1984 and the Quantaphase kit (Bio-Rad laboratories, California) from July to December 1984, cut off <180 pg/mL. Reference standard All abnormalities suggesting deficiency (serum vitamin B12 <180 pg/mL, mean corpuscular volume >99 fL, segmented neutrophil lobe count >3.6/cell, peripheral blood smear interpreted as macrocytic, or abnormal Schilling test result), or fewer abnormalities if the abnormalities lessened in response to parenteral vitamin B12 treatment (1000 µg injection of cyanocobalamin each month). Patients with inconsistent laboratory results or uncertain response to treatment were classified according to the consensus of 2 haematologists who were given all clinical and laboratory data excluding the MMA assay result. Time interval between reference and index test: unclear (categorised as non-deficient if no abnormalities identified at chart review at 6-18 months)
Statistical measures	Outcomes: Clinical deficiency (all abnormalities suggesting deficiency, or fewer abnormalities if abnormalities lessened in response to treatment)

Reference	**Matchar 1987[10]; Matchar 1994[11]**
	Clinical deficiency: urinary MMA (>5 µg/mg creatinine) TP: 7 FP: 1 TN: 88 FN: 0 Sensitivity % 95% CI: 100 (65-100) Specificity% 95% CI: 99 (97-100) PPV % 95% CI: 88 (CI not reported) NPV % 95% CI: 100 (CI not reported) Clinical deficiency: serum vitamin B12 (<180 pg/mL) TP: 16 FP: 56 TN: 62 FN: 0 Sensitivity % 95% CI: 100 (79-100) Specificity% 95% CI: 53 (43-62) PPV % 95% CI: 22.2 (12.6-31.8) NPV % 95% CI: 100 (95.2-100)
Source of funding	Grant from the Veterans Administration Health Service Research and Development Service and from A. W. Mellon Foundation.
Limitations	Risk of bias (QUADAS 2 – risk of bias): serious due to time interval between index test and reference standard Indirectness (QUADAS 2 – applicability): very serious due to mixed age strata, lack of reporting on use of vitamin B12 supplements and lack of a gold standard test for vitamin B12 deficiency
Comments	Sensitivity and specificity values for serum B12 reported in the paper were estimated from the prevalence of abnormal tests in the total population (1,599) and the positive and negative predictive values from the sample of 134. Therefore, sensitivity/specificity values reported above are calculated from TP, FP, TN, FN data.

1

Reference	**Moelby 1990[13]**
Study type	Diagnostic accuracy prospective observational cohort study
Study methodology	Data source: patients undergoing haematological evaluation at a single hospital between April 1988 and April 1989 with serum cobalamin levels <100 pmol l^{-1}. Serum specimens routinely collected by general practitioners at the time of evaluation for cobalamin deficiency.

Reference	**Moelby 1990[13]**
Number of patients	n = 42 Prevalence: 74%
Patient characteristics	Age, range: 24-84 years Pregnancy third trimester: not reported Ethnicity: not reported Gender: 83% female Setting: single hospital Country: Denmark Inclusion criteria: undergoing haematological evaluation for cobalamin deficiency with serum cobalamin levels <100 pmol l^{-1} Exclusion criteria: not reported Vitamin B12 supplements: not reported
Target condition(s)	Vitamin B12 deficiency
Index test(s) and reference standard	Index test (first line): Serum methylmalonic acid measured by a stable isotope dilution technique using solid phase sample extraction and gas chromatography, employing a mass spectrometer in the selected ion-monitoring mode. Cut off >0.34 µmol l^{-1} (3 standard deviations above the mean in a group of normal controls). Reference standard Serum cobalamin concentration <100 pmol l^{-1} and one or both of the following: abnormal Schilling test (cobalamin excretion <10%) and/or megaloblastic bone marrow morphology, which could not be explained by folate deficiency. Time interval between reference and index test: not reported
Statistical measures	Outcomes: Clinical cobalamin deficiency (cobalamin <100 pmol l^{-1} and abnormal Schilling test and/or megaloblastic bone marrow morphology) Clinical deficiency: serum MMA (>0.34 µmol l^{-1}) for TP: 30 FP: 1 TN: 10 FN: 1 Sensitivity % 95% CI: 97 (83-100) Specificity% 95% CI: 91 (59-100) PPV % 95% CI: 97 (CI not reported) NPV % 95% CI: 91 (CI not reported)

Reference	**Moelby 1990[13]**
Source of funding	Grant from the Institute of Experimental Clinical Research, Aarhus University, Denmark.
Limitations	Risk of bias (QUADAS 2 – risk of bias): serious (unclear time interval between reference and index test) Indirectness (QUADAS 2 – applicability): very serious due to mixed age strata, lack of reporting on use of vitamin B12 supplements and lack of a gold standard test for vitamin B12 deficiency
Comments	

1

Reference	**Schrempf 2011[19]**
Study type	Diagnostic accuracy retrospective observational cohort study
Study methodology	Data source: subjects admitted to the Department of Neurology with neuropsychiatric conditions suspicious for VitB12 deficiency between March 2005 and January 2009.
Number of patients	n = 1,279 (only those with normal renal function were included in the main analysis, n=851) Prevalence: 14.8% in total sample (13.2% in those with normal renal function)
Patient characteristics	Age, mean (standard deviation): mixed 67.7 (15.2), range: 18–98 years (65.7 ± 15.2 [18–98] years in those with normal renal function) Pregnancy third trimester: not reported Ethnicity: not reported Gender: 48.9% female (72.7% in the those with normal renal function) Setting: Department of Neurology Country: Germany Inclusion criteria: at least two parameters of the VitB12 status (VitB12, holoTC and/or MMA) Exclusion criteria: not reported Vitamin B12 supplements: data regarding VitB12 supplementation or intake not available Other comments: Indications for screening for VitB12 deficiency were peripheral neuropathy (72.3%), subacute combined degeneration (5.9%), cognitive impairment (8.4%), and other various differential diagnoses for VitB12 deficiency, i.e., multiple sclerosis (13.4%)
Target condition(s)	Vitamin B12 deficiency
Index test(s) and reference standard	<u>Index test (first line):</u> Serum vitamin B12 measured by electrochemiluminescence immunoassay (ECLIA; Modular PPE®, Roche Diagnostics, Grenzach-Wyhlen, Germany, reference from manufacturer: 211–911 pg/ml), cut off <211 pg/ml from manufacturer

Reference	**Schrempf 2011[19]**
	Serum vitamin B12 measured by electrochemiluminescence immunoassay (ECLIA; Modular PPE®, Roche Diagnostics, Grenzach-Wyhlen, Germany, reference from manufacturer: 211–911 pg/ml), cut off <280 pg/ml generated from ROC analyses that provided most approximate sensitivity and specificity Serum vitamin B12 measured by electrochemiluminescence immunoassay (ECLIA; Modular PPE®, Roche Diagnostics, Grenzach-Wyhlen, Germany, reference from manufacturer: 211–911 pg/ml), cut off <395 pg/ml generated from ROC analysis with 95% sensitivity Serum vitamin B12 measured by electrochemiluminescence immunoassay (ECLIA; Modular PPE®, Roche Diagnostics, Grenzach-Wyhlen, Germany, reference from manufacturer: 211–911 pg/ml), cut off <630 pg/ml generated from ROC analysis with 95% sensitivity (whole cohort only) HoloTC measured by microparticle enzyme immunoassay [MEIA, Turbidimetrie (Axsym®, Fa. Abbot, Chicago, USA, reference from manufacturer: 19–119 pmol/l), cut off <19 pmol/l from manufacturer HoloTC measured by microparticle enzyme immunoassay [MEIA, Turbidimetrie (Axsym®, Fa. Abbot, Chicago, USA, reference from manufacturer: 19–119 pmol/l), cut off <42 pmol/l generated from ROC analyses that provided most approximate sensitivity and specificity HoloTC measured by microparticle enzyme immunoassay [MEIA, Turbidimetrie (Axsym®, Fa. Abbot, Chicago, USA, reference from manufacturer: 19–119 pmol/l), cut off <67 pmol/l generated from ROC analysis with 95% sensitivity HoloTC measured by microparticle enzyme immunoassay [MEIA, Turbidimetrie (Axsym®, Fa. Abbot, Chicago, USA, reference from manufacturer: 19–119 pmol/l), cut off <77 pmol/l generated from ROC analysis with 95% sensitivity (whole cohort only) <u>Reference standard</u> MMA > 47 µg/l measured by liquid chromatography tandem mass spectrometry Time interval between reference standard and index test: not reported
Statistical measures	Outcomes: Metabolic vitamin B12 deficiency if MMA > 47 lg/l <u>Metabolic vitamin B12 deficiency (MMA > 47lg/l): serum vitamin B12 (cut off <211 pg/ml) (normal renal function)</u> TP: 27 FP: 61 TN: 401 FN: 44 Sensitivity % 95% CI: 38 (26.7-49.3) Specificity% 95% CI: 86.8 (83.7-89.9) PPV % 95% CI: 30.4 (CIs not reported)

Reference	Schrempf 2011[19]
	NPV % 95% CI: 90.2 (CIs not reported) Metabolic vitamin B12 deficiency (MMA > 47lg/l): serum vitamin B12 (cut off <280 pg/ml) (normal renal function) TP: 47 FP: 175 TN: 287 FN: 24 Sensitivity % 95% CI: 66.2 (55.2-77.2) Specificity% 95% CI: 62.1 (57.7-66.5) PPV % 95% CI: 21 (CIs not reported) NPV % 95% CI: 92.4 (CIs not reported) Metabolic vitamin B12 deficiency (MMA > 47lg/l): serum vitamin B12 (cut off <395 pg/ml) (normal renal function) TP: 64 FP: 298 TN: 164 FN: 7 Sensitivity % 95% CI: 90.1 (83.2-97) Specificity% 95% CI: 35.5 (31.1-39.9) PPV % 95% CI: 17.2 (CIs not reported) NPV % 95% CI: 95.9 (CIs not reported) Metabolic vitamin B12 deficiency (MMA > 47lg/l): serum vitamin B12 (cut off <211 pg/ml) (whole cohort) TP: 38 FP: 77 TN: 555 FN: 73 Sensitivity % 95% CI: 34.2 (25.6-43) Specificity% 95% CI: 87.8 (85.2-90.4) PPV % 95% CI: 32.7 (CIs not reported) NPV % 95% CI: 88.5 (CIs not reported) Metabolic vitamin B12 deficiency (MMA > 47lg/l): serum vitamin B12 (cut off <280 pg/ml) (whole cohort) TP: 70 FP: 225 TN: 407 FN: 41 Sensitivity % 95% CI: 63.1 (54.1-72.1) Specificity% 95% CI: 64.4 (62.7-68.1) PPV % 95% CI: 23.5 (CIs not reported)

Reference	Schrempf 2011[19]
	NPV % 95% CI: 90.9 (CIs not reported) Metabolic vitamin B12 deficiency (MMA > 47lg/l): serum vitamin B12 (cut off <630 pg/ml) (whole cohort) TP: 105 FP: 573 TN: 59 FN: 6 Sensitivity % 95% CI: 94.6 (90.4-98.8) Specificity% 95% CI: 9.3 (7-11.6) PPV % 95% CI: 15.3 (CIs not reported) NPV % 95% CI: 90.8 (CIs not reported) Metabolic vitamin B12 deficiency (MMA > 47lg/l): HoloTC (cut off <19 pmol/l) (normal renal function) TP: 1 FP: 3 TN: 106 FN: 15 Sensitivity % 95% CI: 6.3 (0-18.2) Specificity% 95% CI: 97.2 (94.1-100) PPV % 95% CI: 25.5 (CIs not reported) NPV % 95% CI: 87.2 (CIs not reported) Metabolic vitamin B12 deficiency (MMA > 47lg/l): HoloTC (cut off <42 pmol/l) (normal renal function) TP: 9 FP: 54 TN: 55 FN: 7 Sensitivity % 95% CI: 56.3 (32-80.6) Specificity% 95% CI: 50.5 (41.1-59.9) PPV % 95% CI: 14.7 (CIs not reported) NPV % 95% CI: 88.4 (CIs not reported) Metabolic vitamin B12 deficiency (MMA > 47lg/l): HoloTC (cut off <67 pmol/l) (normal renal function) TP: 14 FP: 93 TN: 15 FN: 2 Sensitivity % 95% CI: 87.5 (71.3-100) Specificity% 95% CI: 13.9 (7.3-19.3)

Reference	Schrempf 2011[19]
	PPV % 95% CI: 13.4 (CIs not reported) NPV % 95% CI: 88 (CIs not reported) <u>Metabolic vitamin B12 deficiency (MMA > 47lg/l): HoloTC (cut off <19 pmol/l) (whole cohort)</u> TP: 1 FP: 4 TN: 143 FN: 23 Sensitivity % 95% CI: 4.2 (0-12.2) Specificity% 95% CI: 97.3 (91.5-99.9) PPV % 95% CI: 21.3 (CIs not reported) NPV % 95% CI: 85.4 (CIs not reported) <u>Metabolic vitamin B12 deficiency (MMA > 47lg/l): HoloTC (cut off <42 pmol/l) (whole cohort)</u> TP: 11 FP: 67 TN: 76 FN: 13 Sensitivity % 95% CI: 45.8 (25.9-65.7) Specificity% 95% CI: 53.1 (44.9-61.3) PPV % 95% CI: 14.5 (CIs not reported) NPV % 95% CI: 84.9 (CIs not reported) <u>Metabolic vitamin B12 deficiency (MMA > 47lg/l): HoloTC (cut off <77 pmol/l) (whole cohort)</u> TP: 23 FP: 129 TN: 13 FN: 1 Sensitivity % 95% CI: 95.8 (87.8-100) Specificity% 95% CI: 9.2 (4.5-13.7) PPV % 95% CI: 15.5 (CIs not reported) NPV % 95% CI: 92.7 (CIs not reported)
Source of funding	The authors declare that they have not received support in the form of grants and/or equipment and drugs from any source.
Limitations	Risk of bias (QUADAS 2 – risk of bias): very serious due to lack of reporting regarding whether index tests and reference standard were conducted and interpreted without knowledge of each other and the time interval between index tests and reference standard and high number of participants excluded from the analysis with little explanation Indirectness (QUADAS 2 – applicability): very serious due to mixed age strata, lack of information regarding use of vitamin B12 supplements and lack of a gold standard test for vitamin B12 deficiency

Reference	**Schrempf 2011**[19]
Comments	Only those with available MMA values were included in the analysis. Authors state no significant differences of VitB12, holoTC or MMA between the various cohorts with all or with incomplete VitB12 parameters available were detected, however it is unclear whether there were differences between those with complete and incomplete MMA data. Main analysis restricted to the cohort with normal renal function, but data on the overall patient cohort are also reported. Separate analysis reported for sub cohorts: clinical syndromes that can clearly result from vitamin B12 deficiency (peripheral neuropathy and/or subacute combined degeneration; n = 649) and peripheral neuropathy as the most important indication for vitamin B12 testing (n = 591)

D.2 Intervention

No evidence identified.

Appendix E – Forest plots

E.1 Diagnostic accuracy - Coupled sensitivity and specificity forest plots

E.1.1 Serum cobalamin assay (first line)

Figure 3: Sensitivity and specificity of serum cobalamin <116 pmol/L for diagnosing deficiency (MMA response to treatment)

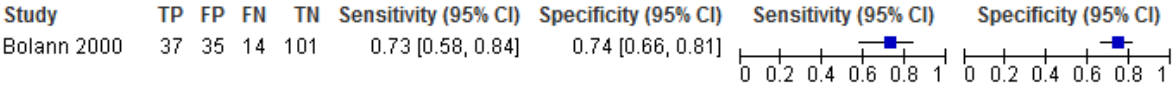


Figure 4: Sensitivity and specificity of serum cobalamin <150 pmol/L for diagnosing deficiency (MMA response to treatment)

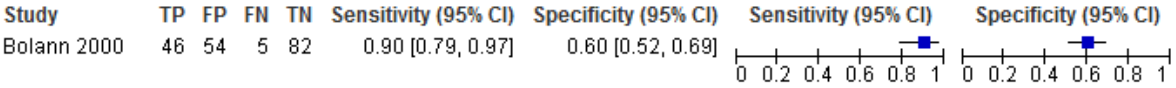


Figure 5: Sensitivity and specificity of B12 <167 pmol/L for diagnosing possible/probable deficiency (4cB12 ≤ -1.5)

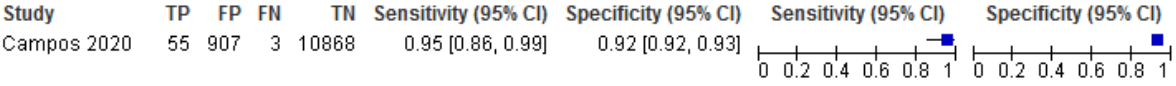


Figure 6: Sensitivity and specificity of B12 <320 pmol/L for diagnosing possible/probable deficiency (4cB12 ≤ -1.5)

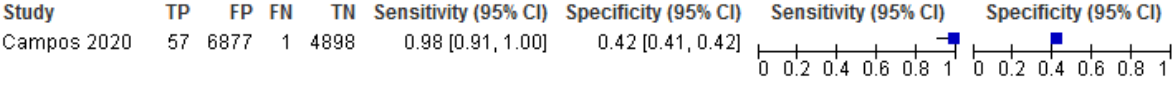


Figure 7: Sensitivity and specificity of B12 <115 pmol/L for diagnosing possible/probable deficiency (4cB12 ≤ -1.5)

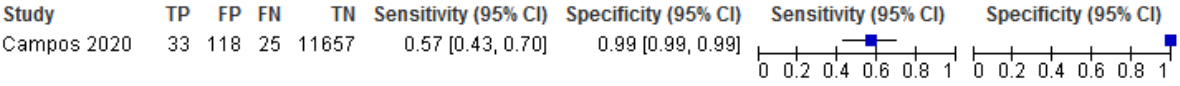
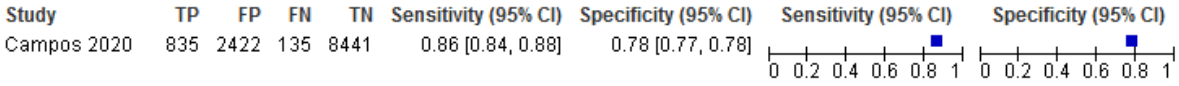
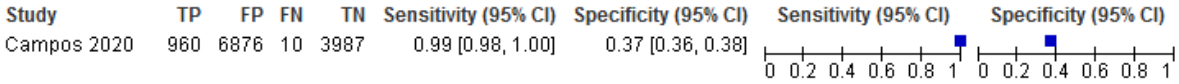


Figure 8: Sensitivity and specificity of B12 <229 pmol/L for diagnosing subclinical deficiency (4cB12 ≤ -0.5 and >-1.5)



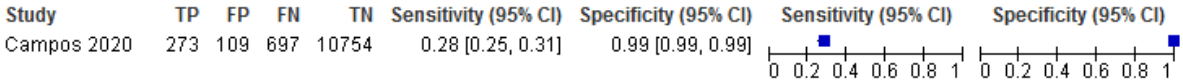
1

Figure 9: Sensitivity and specificity of B12 <351 pmol/L for diagnosing subclinical deficiency (4cB12 ≤ -0.5 and >-1.5)



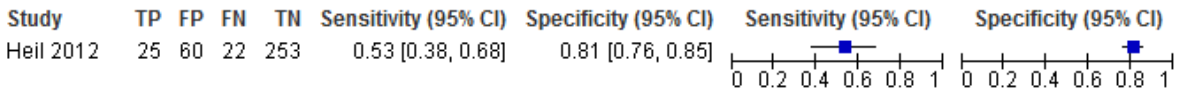
2

Figure 10: Sensitivity and specificity of B12 <142 pmol/L for diagnosing subclinical deficiency (4cB12 ≤ -0.5 and >-1.5)



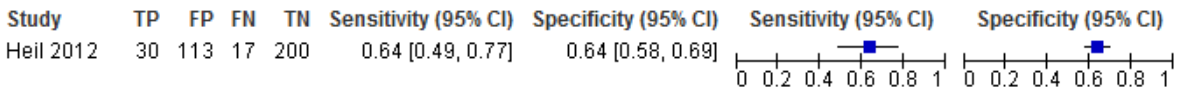
3

Figure 11: Sensitivity and specificity of serum vitamin B12 <145 pmol/L for diagnosing metabolic deficiency (MMA >0.45 µmol/L)



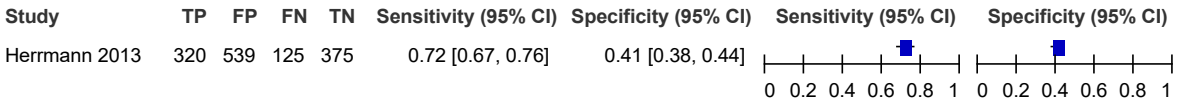
4

Figure 12: Sensitivity and specificity of serum vitamin B12 <180 pmol/L for diagnosing metabolic deficiency (MMA >0.45 µmol/L)



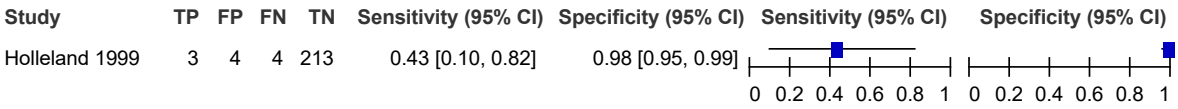
5

Figure 13: Sensitivity and specificity of serum vitamin B12 <227 pM for diagnosing deficiency (MMA >300 nM)



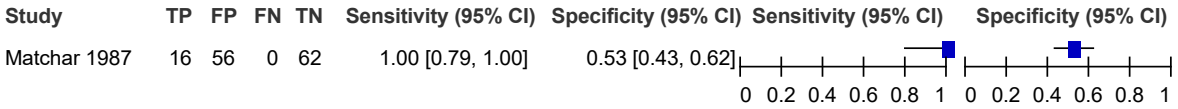
6

Figure 14: Sensitivity and specificity of serum cobalamin ≤170 pmol/L for diagnosing functional deficiency (MMA >0.376 µmol/L)



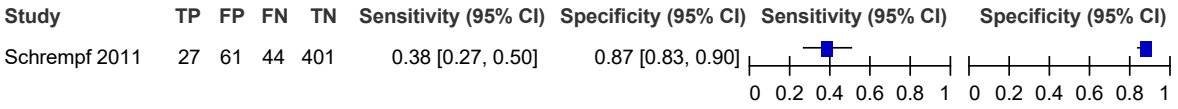
1

Figure 15: Sensitivity and specificity of serum vitamin B12 <180 pg/mL for diagnosing clinical deficiency (all abnormalities suggesting deficiency, or fewer if lessened in response to treatment)



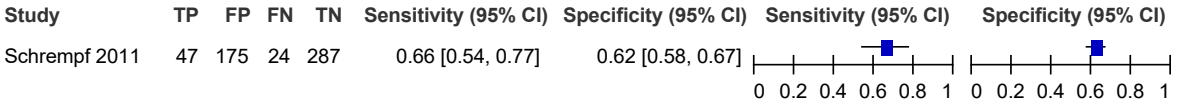
2

Figure 16: Sensitivity and specificity of serum vitamin B12 <211 pg/ml for diagnosing metabolic deficiency (MMA >47 µg/l) (normal renal function)



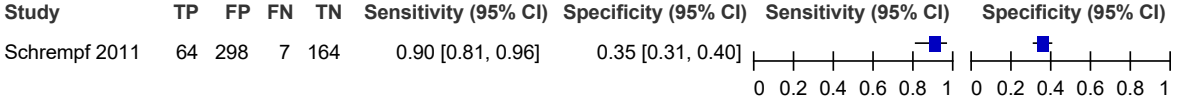
3

Figure 17: Sensitivity and specificity of serum vitamin B12 <280 pg/ml for diagnosing metabolic deficiency (MMA >47 µg/l) (normal renal function)



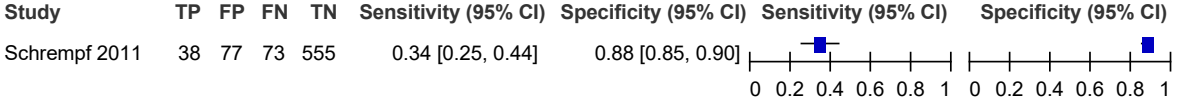
4

Figure 18: Sensitivity and specificity of serum vitamin B12 <395 pg/ml for diagnosing metabolic deficiency (MMA >47 µg/l) (normal renal function)



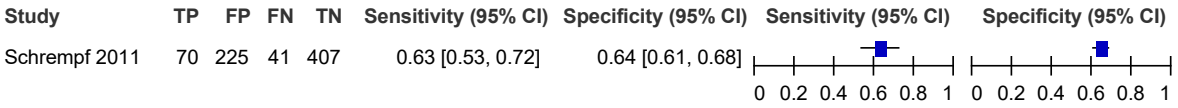
5

Figure 19: Sensitivity and specificity of serum vitamin B12 <211 pg/ml for diagnosing metabolic deficiency (MMA >47 µg/l) (whole cohort)



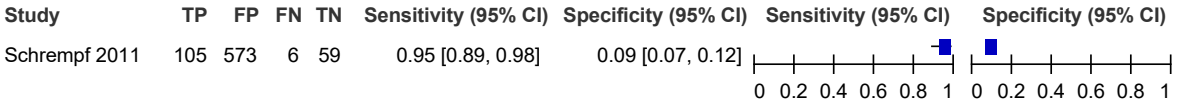
1

Figure 20: Sensitivity and specificity of serum vitamin B12 <280 pg/ml for diagnosing metabolic deficiency (MMA >47 µg/l) (whole cohort)



2

Figure 21: Sensitivity and specificity of serum vitamin B12 <630 pg/ml for diagnosing metabolic deficiency (MMA >47 µg/l) (whole cohort)



3

E.1.21 Holotranscobalamin (first line)

Figure 22: Sensitivity and specificity of holotranscobalamin for diagnosing B12 deficiency (serum B12 <200 pg/mL)

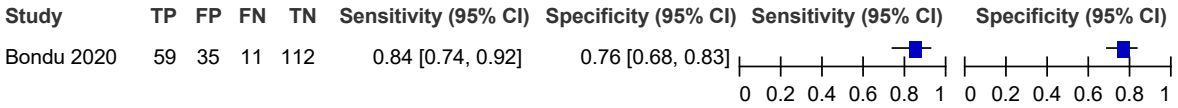


Figure 23: Sensitivity and specificity of holotranscobalamin for diagnosing B12 deficiency and borderline deficiency (serum B12 ≤350 pg/mL)

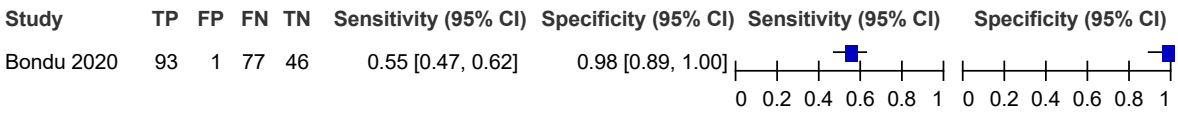


Figure 24: Sensitivity and specificity of holotranscobalamin <27 pmol/L for diagnosing possible/probable deficiency (4cB12 ≤ -1.5)

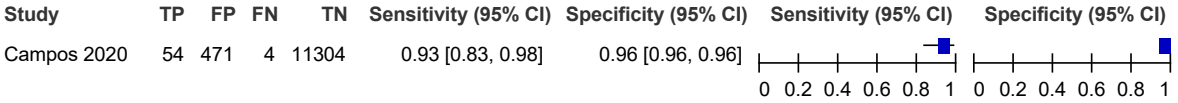


Figure 25: Sensitivity and specificity of holotranscobalamin <56.5 pmol/L for diagnosing possible/probable deficiency (4cB12 ≤ -1.5)

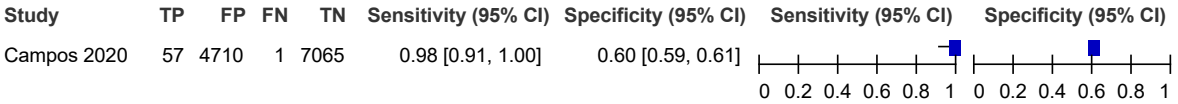


Figure 26: Sensitivity and specificity of holotranscobalamin <19 pmol/L for diagnosing possible/probable deficiency (4cB12 ≤ -1.5)

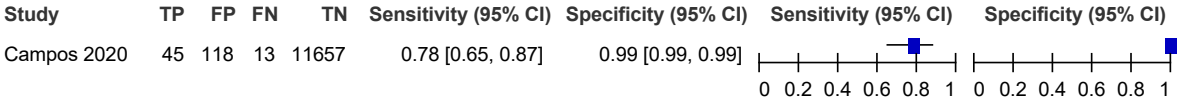
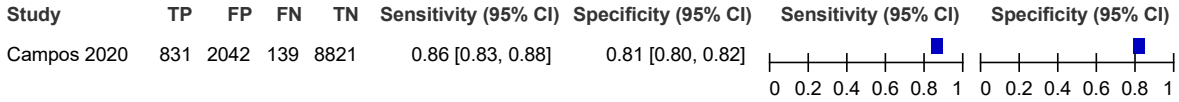
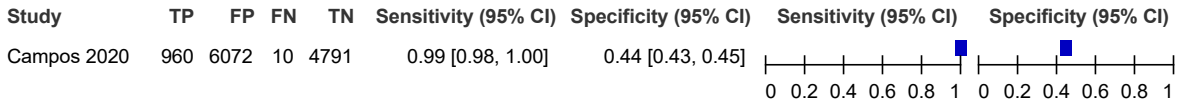


Figure 27: Sensitivity and specificity of holotranscobalamin <45 pmol/L for diagnosing subclinical deficiency (4cB12 ≤ -0.5 and >-1.5)



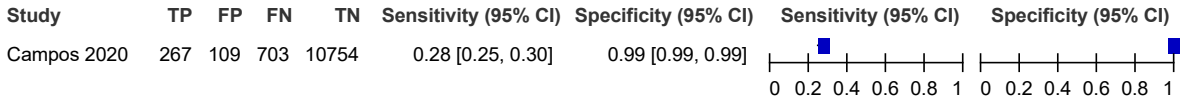
1

Figure 28: Sensitivity and specificity of holotranscobalamin <73 pmol/L for diagnosing subclinical deficiency (4cB12 ≤ -0.5 and >-1.5)



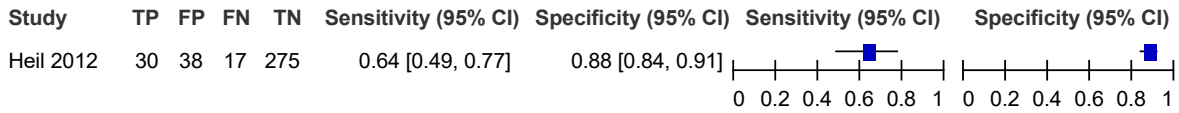
2

Figure 29: Sensitivity and specificity of holotranscobalamin <25 pmol/L for diagnosing subclinical deficiency (4cB12 ≤ -0.5 and >-1.5)



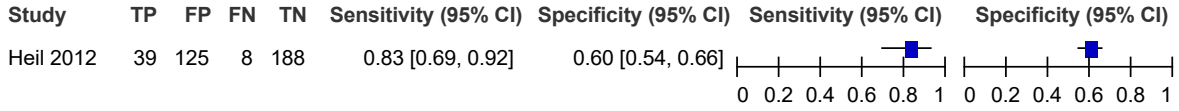
3

Figure 30: Sensitivity and specificity of holotranscobalamin <21 pmol/L for diagnosing metabolic deficiency (MMA >0.45 µmol/)L



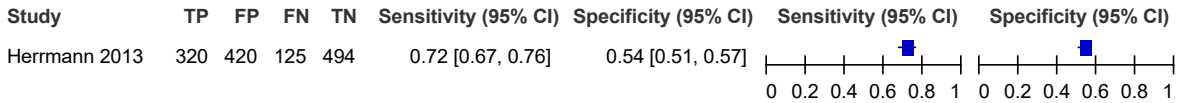
4

Figure 31: Sensitivity and specificity of holotranscobalamin <32 pmol/L for diagnosing metabolic deficiency (MMA >0.45 µmol/)L



5

Figure 32: Sensitivity and specificity of holotranscobalamin <35 pM for diagnosing deficiency (MMA >300 nM)



6

Figure 33: Sensitivity and specificity of holotranscobalamin <22 pM for diagnosing deficiency (MMA >300 nM) (serum creatinine ≤ 97.2 µM))

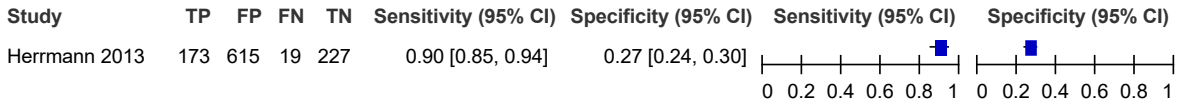


Figure 34: Sensitivity and specificity of holotranscobalamin <76 pM for diagnosing deficiency (MMA >300 nM) (serum creatinine ≤ 97.2 µM))

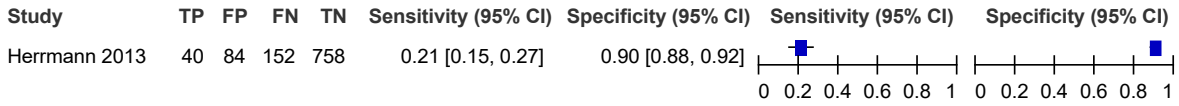


Figure 35: Sensitivity and specificity of holotranscobalamin <19 pmol/l for diagnosing metabolic deficiency (MMA >47 µg/)l (normal renal function)

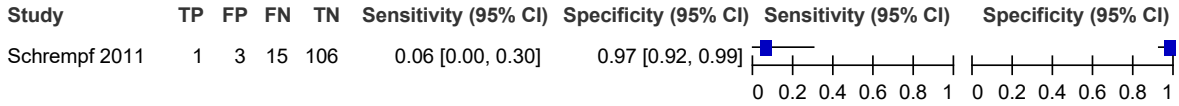


Figure 36: Sensitivity and specificity of holotranscobalamin <42 pmol/l for diagnosing metabolic deficiency (MMA >47 µg/)l (normal renal function)

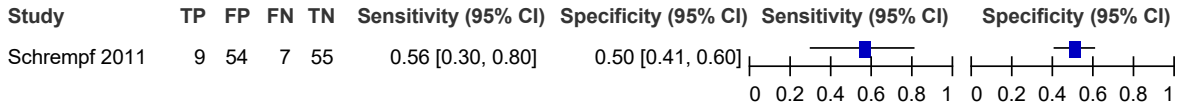


Figure 37: Sensitivity and specificity of holotranscobalamin <67 pmol/l for diagnosing metabolic deficiency (MMA >47 µg/)l (normal renal function)

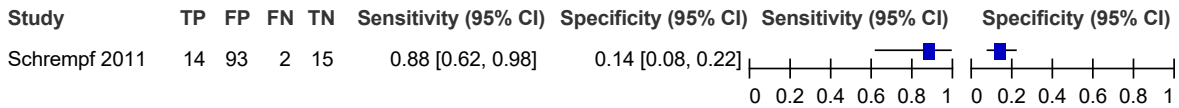


Figure 38: Sensitivity and specificity of holotranscobalamin <19 pmol/l for diagnosing metabolic deficiency (MMA >47 µg/)l (whole cohort)

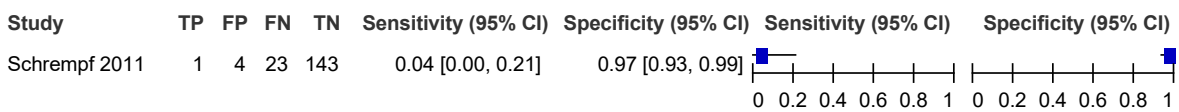


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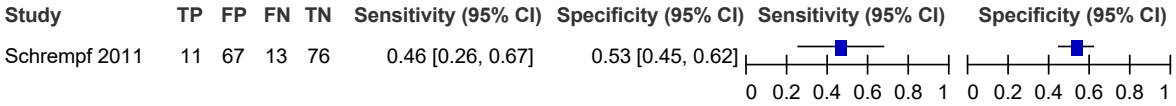
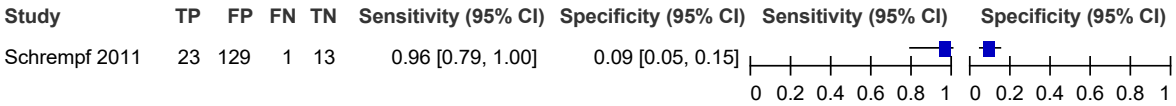
Figure 39: Sensitivity and specificity of holotranscobalamin <42 pmol/l for diagnosing metabolic deficiency (MMA >47 µg/)l (whole cohort)



2

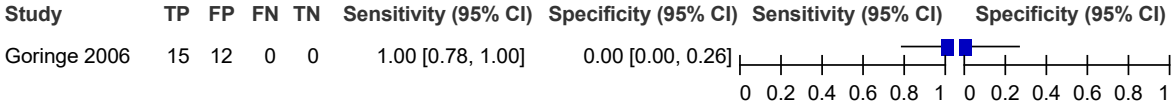
Figure 40: Sensitivity and specificity of holotranscobalamin <77 pmol/l for diagnosing metabolic deficiency (MMA >47 µg/)l (whole cohort)



3

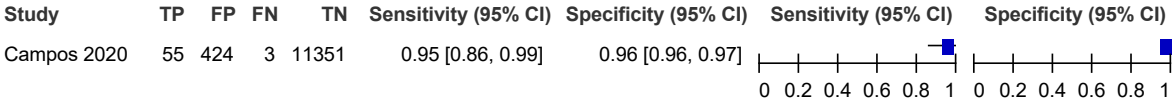
E.1.34 Holotranscobalamin (second line)

Figure 41: Sensitivity and specificity of holotranscobalamin <38 pmol/L for diagnosing response to treatment



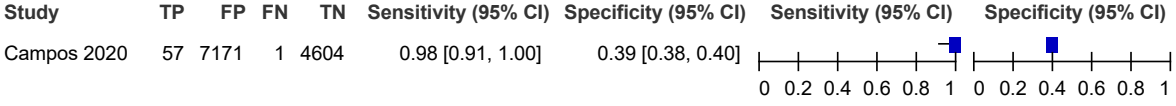
E.1.45 Methylmalonic acid (first line)

Figure 42: Sensitivity and specificity of MMA >466 nmol/L for diagnosing possible/probable deficiency (4cB12 ≤ -1.5)



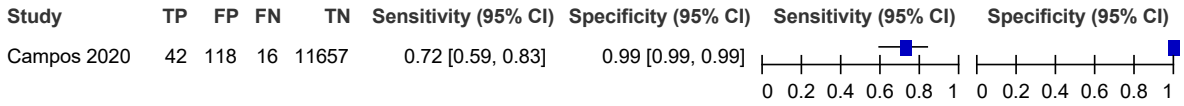
6

Figure 43: Sensitivity and specificity of MMA >158 nmol/L for diagnosing possible/probable deficiency (4cB12 ≤ -1.5)



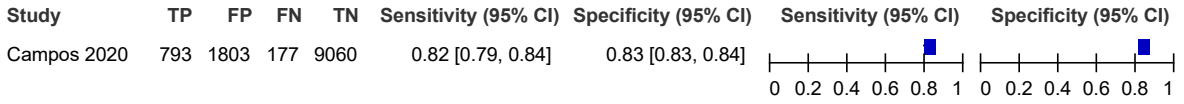
7

Figure 44: Sensitivity and specificity of MMA >723 nmol/L for diagnosing possible/probable deficiency (4cB12 ≤ -1.5)



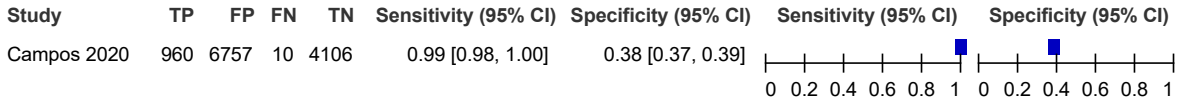
1

Figure 45: Sensitivity and specificity of MMA >245 nmol/L for diagnosing subclinical deficiency (4cB12 ≤ -0.5 and >-1.5)



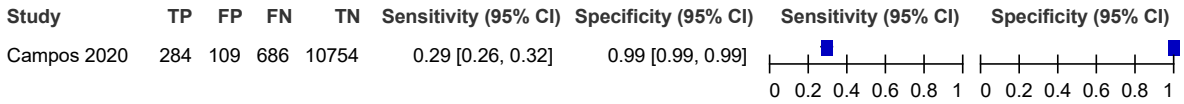
2

Figure 46: Sensitivity and specificity of MMA >152 nmol/L for diagnosing subclinical deficiency (4cB12 ≤ -0.5 and >-1.5)



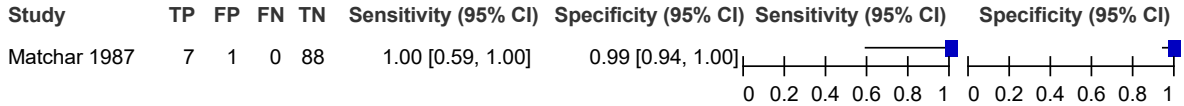
3

Figure 47: Sensitivity and specificity of MMA >480 nmol/L for diagnosing subclinical deficiency (4cB12 ≤ -0.5 and >-1.5)



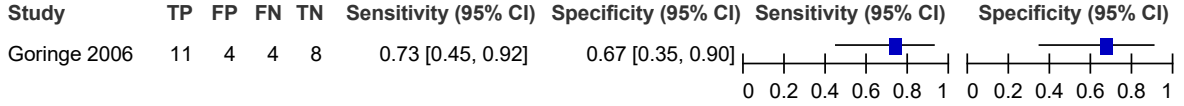
4

Figure 48: Sensitivity and specificity of urinary MMA >5 µg/mg creatinine for diagnosing clinical deficiency (all abnormalities suggesting deficiency, or fewer if lessened in response to treatment)



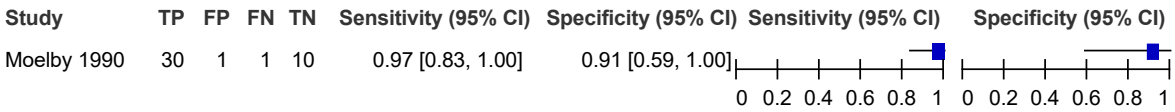
E.1.5 Methylmalonic acid (second line)

Figure 49: Sensitivity and specificity of MMA >0.47 µmol/L for diagnosing response to treatment



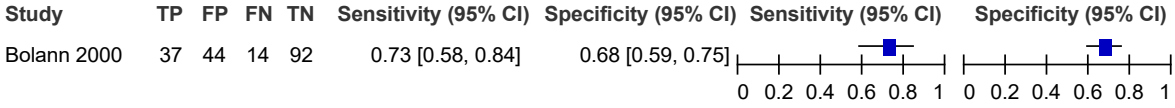
1

Figure 50: Sensitivity and specificity of MMA >0.34 µmol l-1 for diagnosing clinical deficiency (cobalamin <100 pmol l-1 and abnormal Schilling test and/or megaloblastic bone marrow morphology)



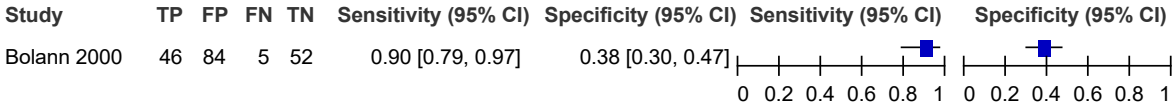
E.1.62 Homocysteine (first line)

Figure 51: Sensitivity and specificity of plasma total homocysteine >15 µmol/L for diagnosing deficiency (MMA response to treatment)



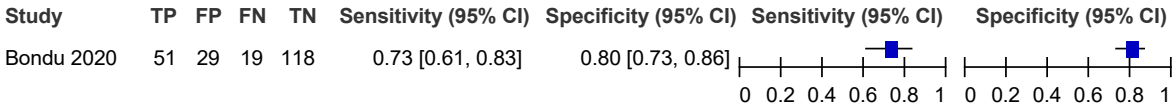
3

Figure 52: Sensitivity and specificity of plasma total homocysteine >11.3 µmol/L for diagnosing deficiency (MMA response to treatment)



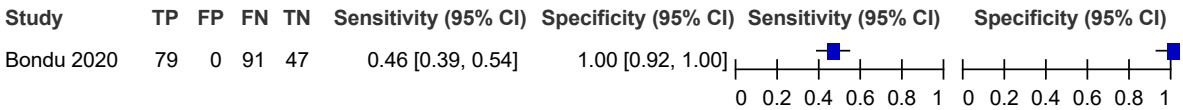
4

Figure 53: Sensitivity and specificity of homocysteine for diagnosing B12 deficiency (serum B12 <200 pg/mL)



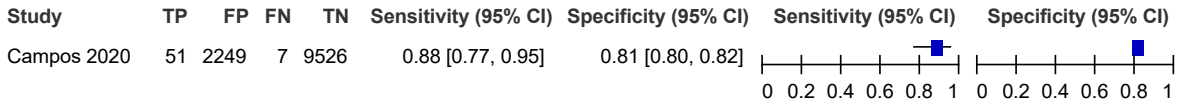
5

Figure 54: Sensitivity and specificity of homocysteine for diagnosing B12 deficiency and borderline deficiency (serum B12 ≤350 pg/mL)



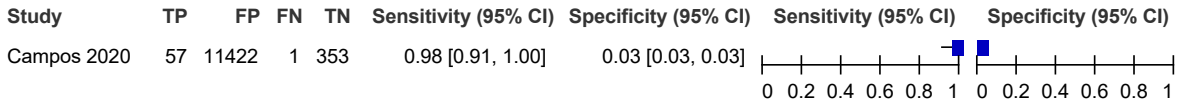
6

Figure 55: Sensitivity and specificity of homocysteine >16.4 µmol/L for diagnosing possible/probable deficiency (4cB12 ≤ -1.5)



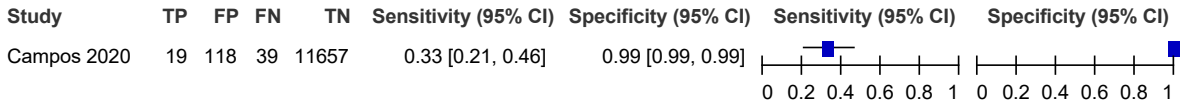
1

Figure 56: Sensitivity and specificity of homocysteine >6.2 µmol/L for diagnosing possible/probable deficiency (4cB12 ≤ -1.5)



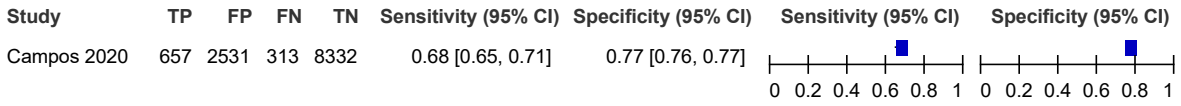
2

Figure 57: Sensitivity and specificity of homocysteine >34 µmol/L for diagnosing possible/probable deficiency (4cB12 ≤ -1.5)



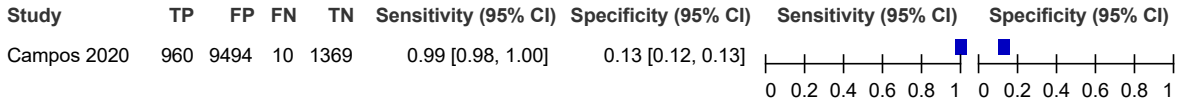
3

Figure 58: Sensitivity and specificity of homocysteine >15 µmol/L for diagnosing subclinical deficiency (4cB12 ≤ -0.5 and >-1.5)



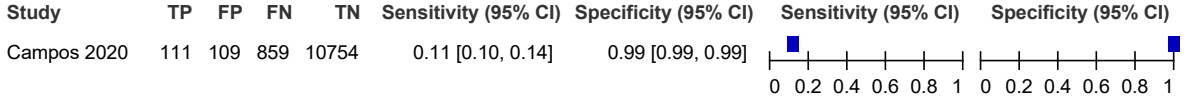
4

Figure 59: Sensitivity and specificity of homocysteine >8 µmol/L for diagnosing subclinical deficiency (4cB12 ≤ -0.5 and >-1.5)



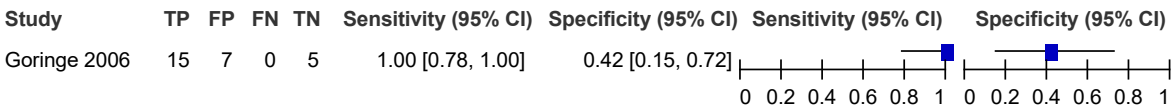
5

Figure 60: Sensitivity and specificity of homocysteine >29 µmol/L for diagnosing subclinical deficiency (4cB12 ≤ -0.5 and >-1.5)



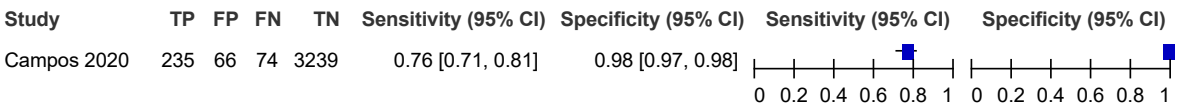
E.1.71 Homocysteine (second line)

Figure 61: Sensitivity and specificity of homocysteine >15 µmol/L for diagnosing response to treatment



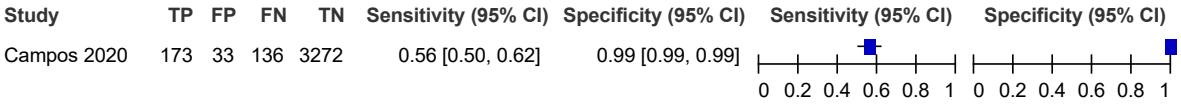
E.1.82 Combinations (first line)

Figure 62: Sensitivity and specificity of 2cB12HoloTC/MMA for diagnosing inadequate B12 status (4cB12 <-0.5)



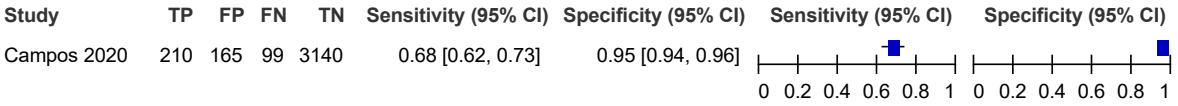
3

Figure 63: Sensitivity and specificity of 2cB12B12/MMA for diagnosing inadequate B12 status (4cB12 <-0.5)



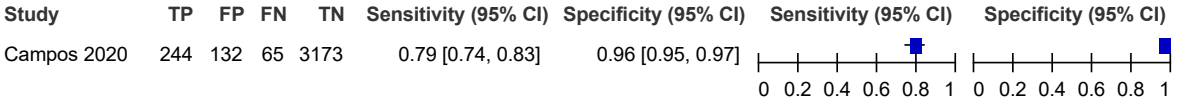
4

Figure 64: Sensitivity and specificity of 2cB12B12/Hcy for diagnosing inadequate B12 status (4cB12 <-0.5)



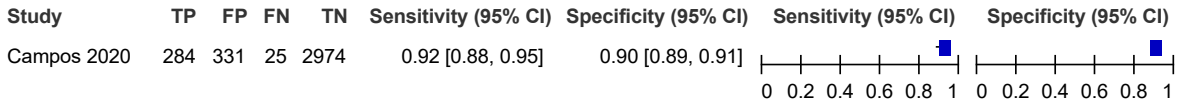
5

Figure 65: Sensitivity and specificity of 2cB12HoloTC/B12 for diagnosing inadequate B12 status (4cB12 <-0.5)



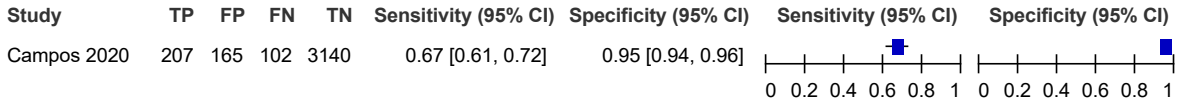
6

Figure 66: Sensitivity and specificity of 2cB12HoloTC/Hcy for diagnosing inadequate B12 status (4cB12 <-0.5)



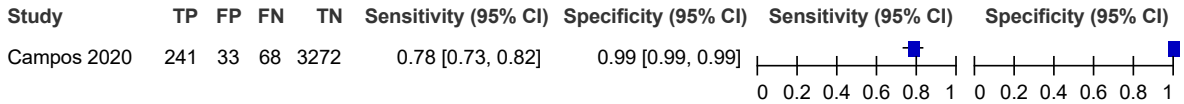
1

Figure 67: Sensitivity and specificity of 2cB12MMA/Hcy for diagnosing inadequate B12 status (4cB12 <-0.5)



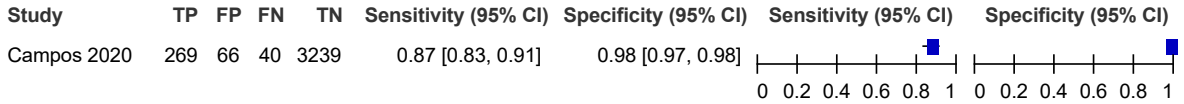
2

Figure 68: Sensitivity and specificity of 3cB12HoloTC/B12/MMA for diagnosing inadequate B12 status (4cB12 <-0.5)



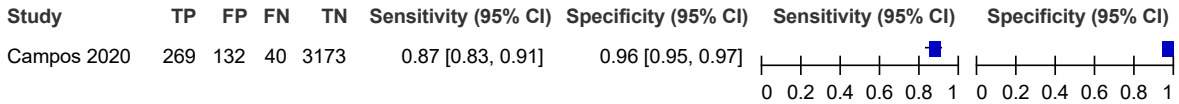
3

Figure 69: Sensitivity and specificity of 3cB12MMA/HoloTC/Hcy for diagnosing inadequate B12 status (4cB12 <-0.5)



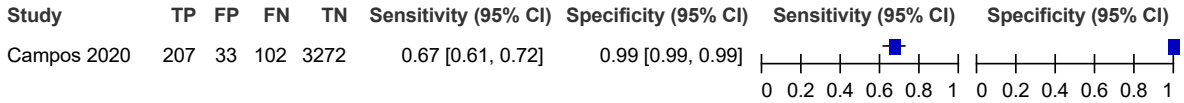
4

Figure 70: Sensitivity and specificity of 3cB12HoloTC/B12/Hcy for diagnosing inadequate B12 status (4cB12 <-0.5)



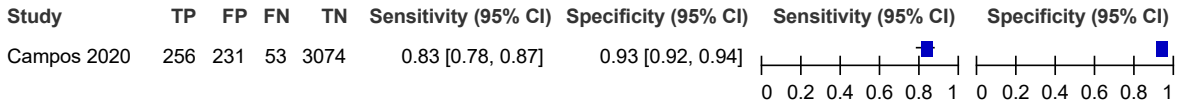
5

Figure 71: Sensitivity and specificity of 3cB12MMA/B12/Hcy for diagnosing inadequate B12 status (4cB12 <-0.5)



E.1.91 Combinations (first and second line)

Figure 72: Sensitivity and specificity of Harrington's algorithm for diagnosing inadequate B12 status (4cB12 <-0.5)



2

E.23 Intervention

4 No forest plots.

5

6

Appendix F – GRADE tables

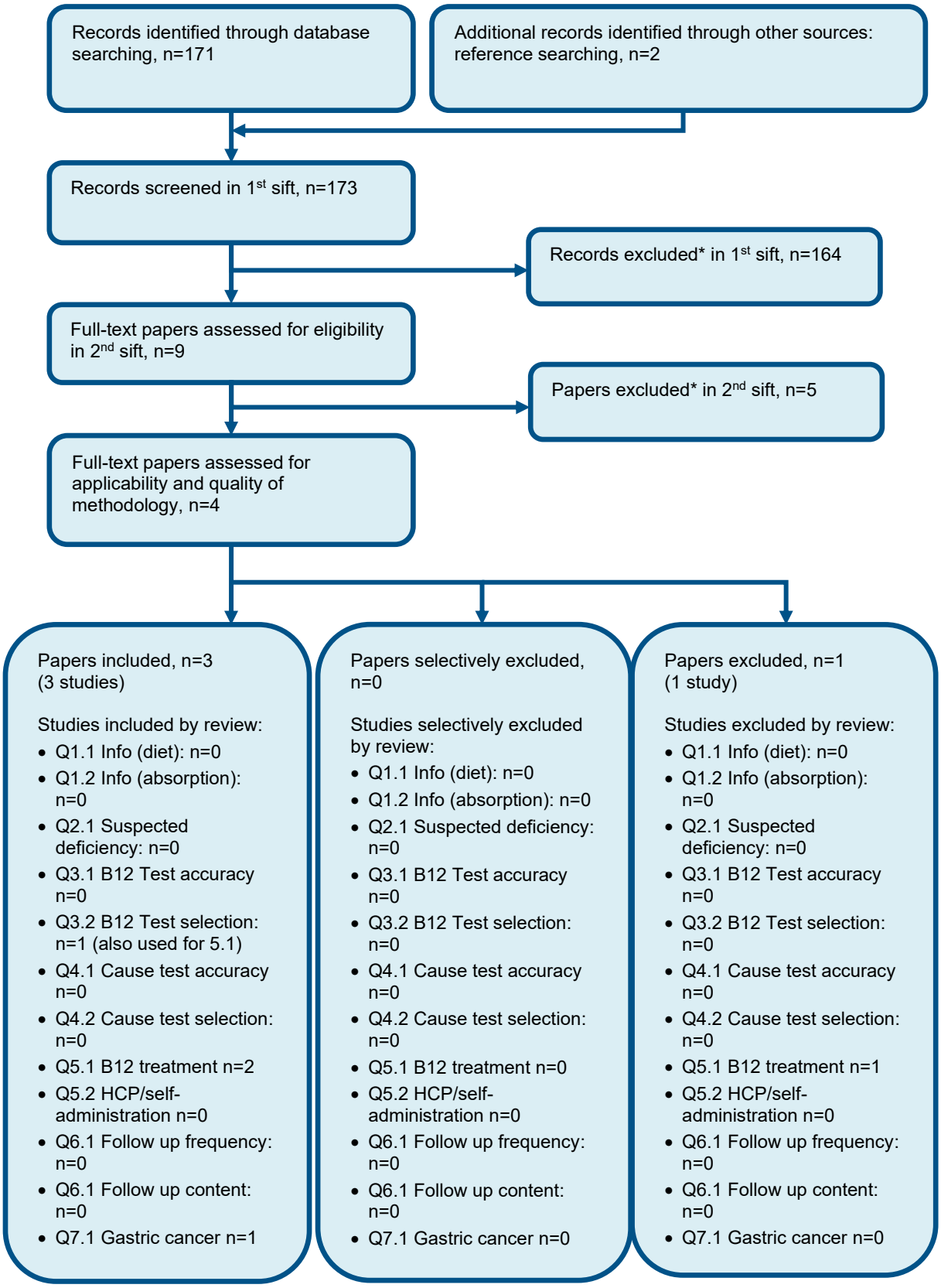
F.1 Diagnostic accuracy

Not applicable

F.2 Intervention

No GRADE tables

Appendix G – Economic evidence study selection



* Non-relevant population, intervention, comparison, design or setting; non-English language

Appendix H – Economic evidence tables

H.1 Diagnostic accuracy

None.

H.2 Intervention

Study	Mnatzaganian, 2015			
Study details	**Population & interventions**	**Costs**	**Health outcomes**	**Cost effectiveness**
Economic analysis: CUA (health outcome: QALYs) **Study design:** Decision Tree **Approach to analysis:** Informed by the guidelines-supported management algorithm, a decision tree comparing five study strategies was developed. The diagnostic test selected was serum cobalamin as this is most used and accessible. **Perspective:** Australia – Medicare **Time horizon:** 3 months **Discounting:** Costs: NA Outcomes: NA	**Population:** 18 years of age or older-newly presenting in general practice with fatigue whose symptoms could not be explained by medical assessment and who had a low pre-test probability of serious illness. **Intervention 1:** Do not test and do no treat; if symptoms continue, reassess after a period of 3 months. **Intervention 2:** Order serum test and treat with Intramuscular Hydroxocobalamin (1,000 µg) nine injections. **Intervention 3:**	**Total costs (mean per patient):** Intervention 1: £65 Intervention 2: £136 Intervention 3: £113 Intervention 4: £221 Intervention 5: £127 **Incremental costs** Intervention 2 – 1 = £71 Intervention 3 – 2 = -£23 Intervention 4 – 3 = £108 Intervention 5 – 4 = £94 **Currency & cost year:** 2013 USA dollars (presented here as 2013 UK pounds[(a)])] **Cost components incorporated:** GP-patient consultation fee, serum cobalamin test, Patient Episode Initiation (specimen) fees,	**QALYs (mean per patient):** Intervention 1: 0.69 Intervention 2: 0.70 Intervention 3: 0.70 Intervention 4: 0.71 Intervention 5: 0.71 **Incremental QALYs** Intervention 2 – 1 = 0.01 Intervention 3 – 2 = 0 Intervention 4 – 3 = 0.01 Intervention 5 – 4 = 0	**ICER** Intervention 2 and intervention 4 are dominated by intervention 5 Intervention 3 is extendedly dominated by intervention 5 Intervention 5 vs intervention 1 £3,105 per QALY gained (pa) 95% CI: NR Probability Intervention 5 cost effective (£20K/£30K threshold): 100% **Analysis of uncertainty:** A pa was performed which included sensitivity and specificity of the diagnostic test, cost, and utility input parameter values. Further deterministic sensitivity analysis was done with varying the prevalence levels of cobalamin deficiency at 1%, 5% 10% and 20%. The sensitivity analyses PSA and one – way, showed that at 1% prevalence the “do not test, do not treat” strategy was the most cost-effective. If prevalence was more than 1% not testing but treating all those presenting with unexplained

	Order serum test and treat with oral supplement (1,000 mcg) – one a day **Intervention 4:** No test, but treat all with IM **Intervention 5:** No test, but treat all with oral supplement	medication costs, service costs for IM injections.		fatigue with oral supplements was the most cost effective strategy.
Data sources				
Health outcomes: The sensitivity and specificity of the serum cobalamin test derived from SR and meta-analysis of studies (Willis et al., 2011). Effectiveness of medication – RCT/SR. **Quality-of-life weights:** EQ-5D utility score derived without using UK-tariff but using hypothetical state scenarios from an Australian perspective. **Cost sources:** Pharmaceutical Benefits Scheme fees, Medicare Australia				
Comments				
Source of funding: National Health and Medical Research Council of Australia **Limitations:** This study doesn't compare the different available diagnostic tests for b12 deficiency. However, it focuses on diagnosis and intervention together using serum cobalamin testing with oral and IM treatment. Fatigue is only one symptom which may be related to B12 deficiency / pernicious anaemia, so this study does not capture all potential B12 deficient people. Only the first consultation was considered; all screening tests that could have been ordered—other than serum cobalamin—were not included in this economic evaluation. The cost of misdiagnosis/referral to specialists was not included which could have a significant impact of the cost-effectiveness. The UK tariff was not used for EQ-5D; The utility scores were derived using hypothetical state scenarios which may not be comparable to one obtained by using patient data. There is uncertainty regarding the baseline prevalence of B12 deficiency. In addition to that the time horizon of three months may not be adequate and the risk of recurrence of deficiency or symptoms after three months were not explored. **Other:**				
Overall applicability:[(b)] Partially applicable **Overall quality:**[(c)] Potentially serious limitations				

Abbreviations: 95% CI= 95% confidence interval; CUA= cost–utility analysis; da= deterministic analysis; EQ-5D= Euroqol 5 dimensions (scale: 0.0 [death] to 1.0 [full health], negative values mean worse than death); ICER= incremental cost-effectiveness ratio; NR= not reported; IM= intramuscular; mcg = microgram; pa= probabilistic analysis; QALYs= quality-adjusted life years

(a) Converted using [2013] purchasing power parities[18]
(b) Directly applicable / Partially applicable / Not applicable
(c) Minor limitations / Potentially serious limitations / Very serious limitations

Appendix I – Health economic model

I.1 Model specification

Population: People who have had an indeterminate result with either an active B12 test or total B12 test

Comparison: ‘MMA testing, treat positive’ vs ‘No MMA testing, treating all’ vs ‘No MMA testing, no treatment’

Perspective: National Health Service and Personal Social Services.

Outcomes: Quality-adjusted life-years (QALYs).

I.2 Model inputs and methods

Model approach

A decision tree model was developed using expert opinion from committee members and published data. An overview of the model structure is displayed below.

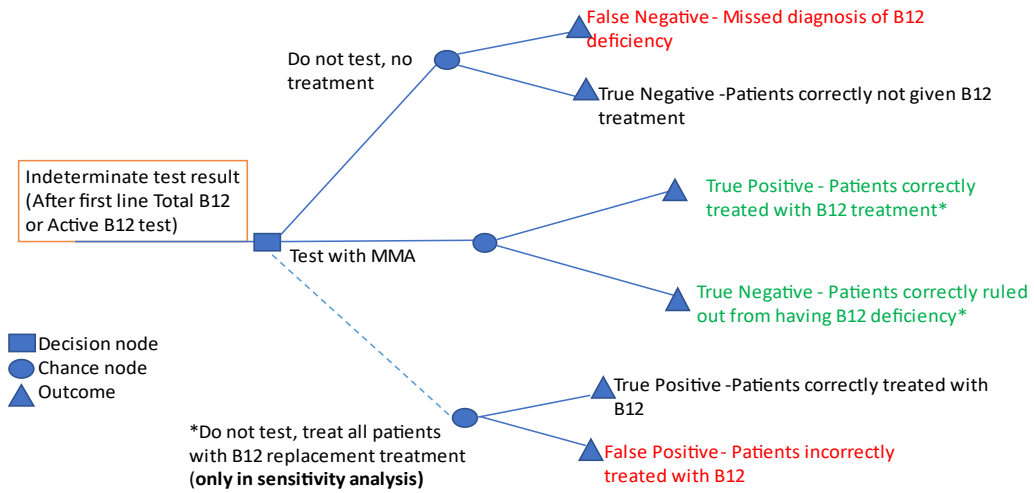


Figure 73 – schematic overview of the MMA decision model for people who present with a first-line indeterminate test result.

There are alternative management options for people that present with an indeterminate test result. These strategies are 'do not test, no treatment' or to test with MMA. An alternative strategy 'do not test, treat all' was considered in a sensitivity analysis. People that present with an indeterminate result are assumed to either be B12 deficient or to have self-limiting transient symptoms that resolve within three months. The proportions of these groups are taken from the prevalence of B12 deficiency which is 24.3% of the population that have a first line indeterminate test result with a range of 14% to 40%.

A treatment effect in terms of improvement in utility was applied to people that are correctly given B12 treatment (i.e., because they have B12 deficiency) which is represented by the true positives in the above schematic (figure 74). This also applied to the true negatives and false positives as these people do not have an underlying B12 deficiency but have self-limiting transient symptoms (so that their utility improves regardless of B12 treatment).

People who have B12 deficiency but were not given any treatment show no improvement in their utility, and it is assumed that they will present again to primary care for a further GP appointment. This is represented by the false negatives in the 'do not test, no treatment' strategy in the above schematic (figure 75),**Characteristics of MMA testing (accuracy)**

In the absence of any further clinical evidence or any better reference standard, MMA testing is assumed to be 100% accurate in terms of sensitivity and specificity. This means that every person tested positive is assumed to have B12 deficiency and every person tested negative is assumed to have transient symptoms and no B12 deficiency.

Prevalence of underlying B12 deficiency in the population

The prevalence of B12 deficiency was taken from Sobczyńska-Malefora (2015). The study reported that there were 9073 people (42.9% male) attending hospital wards and outpatient clinics who were tested for B12 status as a routine test using active B12. For people who had an active B12 of between 25-70 pmol/L, an MMA test was conducted. There was a total of 3,122 samples which were initially tested for active B12 and then tested with MMA which provides data of B12 prevalence.

Treatment effects for people that have B12 deficiency.

People who have B12 deficiency and are offered B12 treatment will achieve health gains that would not be realised without treatment. For people who do not have B12 deficiency it is assumed that their symptoms are transient, and their health gains will be identical to the health gains of a treated B12 deficient person. For people that have B12 deficiency but who are not offered treatment their utility will remain at baseline and will not improve. Utility data for the economic model were taken from Mnatzaganian et al. (2015) (see Table 21). The calculation of utility scores was based on the assignment levels of for each of the EuroQoL-5D (EQ-5D) attributes. To estimate the EQ-5D attributes the authors conducted a systematic review on fatigue prognosis combined with expert opinion. The utility data published was only limited to 3 months post baseline, however assuming correct treatment it is assumed the utility will remain the same. In the decision model, a linear transformation of utility between timepoints is assumed. The QALYs are equal to the area under the curve for utility with respect to time.

Table 21: Utility data

	Description of assumed utility values	Treated	Untreated
Baseline	No problems walking about / No problems with self care / Some problems performing usual activities / Moderate pain or discomfort / Moderately anxious or depressed	0.689	0.689
1 month post baseline	No problems walking about / No problems with self care / Some problems performing usual activities / Moderate pain or discomfort / Not anxious or depressed	0.76	0.689
2 months post baseline	No problems walking about / No problems with self care / Some problems performing usual activities / No pain or discomfort / Moderately anxious or depressed	0.833	0.689
3 months and onwards post baseline	Well	1	0.689

Intervention costs

The cost of the MMA test (£30) was taken as an average of the costs obtained by committee members in their practices.

Although for Orobalin the licensed treatment dose is initially 4000mcg daily until remission, there is uncertainty about how long the time taken to remission is and how to assess remission which may be evaluated by further B12 tests or assessment of a person's symptoms. The experience of the committee is that for newly diagnosed people with B12 deficiency, when cyanocobalamin 1000mcg tablets are prescribed the starting dose is one tablet a day rather than four tablets a day. Therefore, treatment was assumed to be cyanocobalamin (Orobalin) 1mg/day as this is assumed to be more effective than the 50mcg/day cyanocobalamin dose, whilst being a lower cost than the 50mcg form. The cost is £9.99 per 30 tablets with the dose assumed to be one tablet per day.

For people who are not offered MMA testing, the costs of complications and hospitalisations due to B12 deficiency were not incorporated in the economic analysis due to the uncertainty of the diagnostic/treatment pathway if B12 diagnosis is missed. The cost penalty for a missed B12 deficiency diagnosis is assumed to be one GP appointment (£33).

I.3 Summary of model parameters

The parameters used in the base case analysis are listed in Table 22 which also include ranges used for sensitivity analysis.

Table 22: Overview of parameters in the model

Input	Data	Source
Perspective	UK NHS & personal social services	Developing NICE guidelines: the manual.[15]
Time horizon	3 months (up to 1 year)	Committee members' advice
Discount rate	0%	Considered not relevant because of the short time horizon.
Baseline prevalence		
Prevalence of elevated MMA	24.3% (14% - 40%)	(Sobczynska-Malefora, 2014)[20]
Health-related quality of life (utilities)		
Baseline	0.689	(Mnatzaganian, 2015)[12]
1 month	0.760	(Mnatzaganian, 2015)[12]
2 months	0.883	(Mnatzaganian, 2015)[12]
3 months post baseline – Full health	1.0	(Mnatzaganian, 2015)[12]
Costs		
MMA test	£30.35 (£11.00 - £49.41)	Committee members' advice
9.22 min GP appointment cost	£33	Unit costs of health and social care 2021[9]
Treatment – Orobalin 1mg	£9.99 per month	NHS electronic drug tariff[16]

I.4 Results

The incremental cost of 'MMA testing' vs 'no MMA testing, no treatment' was £29.62 per person in the base case analysis whilst the incremental QALYs gained were 0.0075 per person. Therefore the cost per QALY gained from 'MMA testing, treat positive' vs 'no MMA testing, no treatment' was £3,946 in the base case analysis and it was below £20,000 in all the sensitivity analyses conducted. The main results are presented in 1.2.8 above.

Sensitivity analysis with 'no MMA testing, treat all' strategy.

For the MMA vs 'no MMA testing, treat all' strategy, a comparison of costs was conducted. There is assumed to be no difference in health outcomes as all B12 deficient patients are given the appropriate treatment therefore the QALYs are identical in both strategies, and so only costs are presented

Assuming the same parameters as the base case, 'no MMA testing, treating all' MMA testing was less costly. 'MMA testing, treat positive' was less costly (and therefore more cost effective) than 'treat all' in the following scenarios (see Table 23):

- All scenarios with the lower estimate of MMA cost.
- All scenarios with the time horizon (and hence the treatment period) of 12 months.
- The average MMA cost, low prevalence and the time horizon (and hence the treatment period) was 4 months or longer.
- The average MMA cost, mean prevalence and the time horizon (and hence the treatment period) was 5 months or longer.
- The average MMA cost, high prevalence and the time horizon (and hence the treatment period) was 6 months or longer.
- The higher MMA cost and low prevalence and the time horizon was 6 months or longer.

Table 23: Sensitivity analyses (deterministic) – Incremental cost per person, MMA testing, treat positive vs No MMA testing, treat all

Time horizon	Low MMA cost, low prevalence	Low MMA cost, average prevalence	Low MMA cost, high prevalence	Average MMA cost, low prevalence	Average MMA cost, average prevalence	Average MMA cost, high prevalence
3 months	-£14.77	-£11.68	-£6.98	£4.58	*£7.67	£12.37
4 months	-£23.37	-£19.25	-£12.98	-£4.01	£0.11	£6.38
5 months	-£31.96	-£26.81	-£18.97	-£12.60	-£7.45	£0.38
6 months	-£40.55	-£34.37	-£24.96	-£21.20	-£15.02	-£5.61
12 months	-£92.10	-£79.74	-£60.93	-£72.74	-£60.38	-£41.58

* Base case. Please note that the QALYs are the same for each strategy therefore only incremental costs are presented.

Appendix J – Excluded studies

J.1 Diagnostic accuracy

Clinical studies

Table 24: Studies excluded from the clinical review

Study	Code [Reason]
Abd El Dayem, S. M., Saleh, O. N., Emara, N. A. et al. (2014) Evaluation of Homocysteine, folic acid and vitamin b12 levels among Egyptian children with idiopathic epilepsy. Macedonian Journal of Medical Sciences 7(1): 109-113	- Population not relevant to this review protocol
Al Aisari, F.; Al-Hashmi, H.; Mula-Abed, W.-A. (2010) Comparison between serum holotranscobalamin and total vitamin B12 as indicators of vitamin B12 status. Oman Medical Journal 25(1): 9-12	- No reference standard
Ales, J. M. and Vivanco, F. (1954) Vitamin B12 assay in the blood of patients and normal subjects. Bulletin of the Institute for Medical Research, University of Madrid 7(1): 33-9	- No diagnostic accuracy measures
Allen, L. H. and Casterline, J. (1994) Vitamin B-12 deficiency in elderly individuals: Diagnosis and requirements. American Journal of Clinical Nutrition 60(1): 12-14	- Commentary article
Allen, R. H., Stabler, S. P., Savage, D. G. et al. (1992) New approaches to the diagnosis of cobalamin (Cbl, vitamin B12) deficiency in neuropsychiatric disorders. Journal of Nutritional Science & Vitaminology specno: 130-3	- Commentary article
Allen, R. H., Stabler, S. P., Savage, D. G. et al. (1990) Diagnosis of cobalamin deficiency I: usefulness of serum methylmalonic acid and total homocysteine concentrations. American Journal of Hematology 34(2): 90-8	- Full text paper not available
Anonymous (1969) Screening for vitamin-B12 deficiency. Lancet 2(7615): 309-10	- Review article but not a systematic review
Anonymous (1979) Macrocytosis, mild anemia and delay in the diagnosis of pernicious anemia. Nutrition Reviews 37(2): 47-48	- Study design not relevant to this review protocol
Anonymous (2017) Preserving the nerves: Detecting and treating vitamin B12 deficiency in risk patients. Deutsche Apotheker Zeitung 157(22)	- Study not reported in English
Ao, M., Tsuji, H., Shide, K. et al. (2017) High prevalence of vitamin B-12 insufficiency in patients with Crohn's disease. Asia Pacific Journal of Clinical Nutrition 26(6): 1076-1081	- Data not reported in an extractable format or a format that can be analysed
Bain, B., Broom, G. N., Woodside, J. et al. (1982) Assessment of a radioisotopic assay for vitamin B12 using an intrinsic factor preparation with R proteins blocked by vitamin B12	- Study aiming to diagnose malabsorption, not B12 deficiency

Study	Code [Reason]
analogues. Journal of Clinical Pathology 35(10): 1110-3	
Bamonti, F., Moscato, G. A., Novembrino, C. et al. (2010) Determination of serum holotranscobalamin concentrations with the AxSYM active B(12) assay: cut-off point evaluation in the clinical laboratory. Clinical Chemistry & Laboratory Medicine 48(2): 249-53	- Population not relevant to this review protocol
Barness, L. A. (1967) Vitamin B12 deficiency with emphasis on methylmalonic acid as a diagnostic aid. American Journal of Clinical Nutrition 20(6): 573-82	- Review article but not a systematic review
Bohn Stafleu van, Loghum (2015) Diagnosis of vitamin B12 deficiency. Huisarts en Wetenschap 58(12): 666	- Conference abstract
Boutin, M., Presse, N., Martineau, T. et al. (2020) Mass spectrometry analysis of urinary methylmalonic acid to screen for metabolic vitamin B12 deficiency in older adults. Bioanalysis 12(10): 693-705	- Population not relevant to this review protocol
Brady, J., Wilson, L., McGregor, L. et al. (2008) Active B12: a rapid, automated assay for holotranscobalamin on the Abbott AxSYM analyzer. Clinical Chemistry 54(3): 567-73	- Comparator in study does not match that specified in this review protocol
Briedis, D., McIntyre, P. A., Judisch, J. et al. (1973) An evaluation of a dual-isotope method for the measurement of vitamin B 12 absorption. Journal of Nuclear Medicine 14(3): 135-41	- Study aiming to diagnose malabsorption, not B12 deficiency
Britt, R. P., Bolton, F. G., Cull, A. C. et al. (1969) Experience with a simplified method of radio-isotopic assay of serum vitamin B 12. British Journal of Haematology 16(5): 457-64	- No diagnostic accuracy measures
Carlmark, B. and Reizenstein, P. (1974) Comparison of methods to diagnose deficiency or malabsorption of vitamin B12. Scandinavian Journal of Gastroenterology - Supplement 29: 39-42	- Review article but not a systematic review
Chanarin, I. (1987) How to diagnose (and not misdiagnose) pernicious anaemia. Blood Reviews 1(4): 280-3	- Review article but not a systematic review
Chanarin, I. and Metz, J. (1997) Diagnosis of cobalamin deficiency: the old and the new. British Journal of Haematology 97(4): 695-700	- Review article but not a systematic review
Chen, I. W., Silberstein, E. B., Maxon, H. R. et al. (1981) Clinical significance of serum vitamin B12 measured by radioassay using pure intrinsic factor. Journal of Nuclear Medicine 22(5): 447-51	- Comparator in study does not match that specified in this review protocol
Choi, YK; Lee, BJ; Lee, DI (1994) Effects of Nitrous Oxide on Serum Vitamin B12, Folate and Hematopoiesis in Surgical Patients. Korean journal of anesthesiology 27(10): 1300-1308	- Study not reported in English
Chong, Y. H. and Lopez, C. G. (1968) A rapid method for the detection of vitamin B12 deficiency. Medical Journal of Malaya 22(3): 250	- Abstract only

Study	Code [Reason]
Christenson, R. H.; Dent, G. A.; Tuszynski, A. (1985) Two radioassays for serum vitamin B12 and folate determination compared in a reference interval study. Clinical Chemistry 31(8): 1358-60	- Population not relevant to this review protocol
Chu, R. C. and Hall, C. A. (1988) The total serum homocysteine as an indicator of vitamin B12 and folate status. American Journal of Clinical Pathology 90(4): 446-9	- No diagnostic accuracy measures
Chui, C. H., Lau, F. Y., Wong, R. et al. (2001) Vitamin B12 deficiency--need for a new guideline. Nutrition 17(1112): 917-20	- Study design not relevant to this review protocol
Cinemre, H., Serinkan Cinemre, B. F., Cekdemir, D. et al. (2015) Diagnosis of vitamin B12 deficiency in patients with myeloproliferative disorders. Journal of Investigative Medicine 63(4): 636-40	- Population not relevant to this review protocol
Clarke, R., Sherliker, P., Hin, H. et al. (2007) Detection of vitamin B12 deficiency in older people by measuring vitamin B12 or the active fraction of vitamin B12, holotranscobalamin. Clinical Chemistry 53(5): 963-70	- Population not relevant to this review protocol
Cooper, B. A.; Fehedy, V.; Blanshay, P. (1986) Recognition of deficiency of vitamin B12 using measurement of serum concentration. Journal of Laboratory & Clinical Medicine 107(5): 447-52	- No diagnostic accuracy measures
Cooper, B.; Frenkel, E. P.; Colman, N. (1979) Multi-laboratory evaluation of 'serum vitamin B12 level' measured by radioassay for 'total B12' vs. 'true cobalamin' vs. microbiologic assay with Euglena gracilis. Clinical Chemistry 25(6): no369	- Full text paper not available
Cravens, D. D.; Nashelsky, J.; Oh, R. C. (2007) Clinical inquiries. How do we evaluate a marginally low B12 level?. Journal of Family Practice 56(1): 62-3	- Review article but not a systematic review
Curtis, D., Sparrow, R., Brennan, L. et al. (1994) Elevated serum homocysteine as a predictor for vitamin B12 or folate deficiency. European Journal of Haematology 52(4): 227-32	- Population not relevant to this review protocol
Dale, R. A. (1972) The assay of methylmalonic acid in urine. Clinica Chimica Acta 41: 141-7	- Full text paper not available
Dastidar, R. and Sikder, K. (2022) Diagnostic reliability of serum active B12 (holo-transcobalamin) in true evaluation of vitamin B12 deficiency: Relevance in current perspective. BMC research notes 15(1): 329	- Data not reported in an extractable format or a format that can be analysed
Dastidar, Rinini and Sikder, Kunal (2022) Diagnostic reliability of serum active B12 (holo-transcobalamin) in true evaluation of vitamin B12 deficiency: Relevance in current perspective. BMC research notes 15(1): 329	- Duplicate reference
Davis, E. T., Strogach, I., Carobene, M. et al. (2020) Paradoxical Elevation of Both Serum B12 and Methylmalonic Acid Levels in Assessing B12 Status in Children With Short-Bowel	- Study design not relevant to this review protocol

Study	Code [Reason]
Syndrome. Jpen: Journal of Parenteral & Enteral Nutrition 44(7): 1257-1262	
Dawson, D. W. (1984) Diagnosis of vitamin B12 deficiency. British Medical Journal 289(6450): 938-939	- Review article but not a systematic review
Dias, S.; Gonther, V.; Suter, P. M. (2016) CME: Differential diagnosis of elevated plasma vitamin B12 levels. Praxis 105(17): 995-1000	- Study not reported in English
Elin, R. J. and Winter, W. E. (2001) Methylmalonic acid: a test whose time has come?. Archives of Pathology & Laboratory Medicine 125(6): 824-7	- Review article but not a systematic review
Fairbanks, V. F. and Elveback, L. R. (1983) Tests for pernicious anemia: serum vitamin B12 assay. Mayo Clinic Proceedings 58(2): 135-7	- Review article but not a systematic review
Fedosov, S. N. (2010) Metabolic signs of vitamin B(12) deficiency in humans: computational model and its implications for diagnostics. Metabolism: Clinical & Experimental 59(8): 1124-38	- Comparator in study does not match that specified in this review protocol
Fedosov, S. N., Brito, A., Miller, J. W. et al. (2015) Combined indicator of vitamin B12 status: modification for missing biomarkers and folate status and recommendations for revised cut-points. Clinical Chemistry & Laboratory Medicine 53(8): 1215-25	- No diagnostic accuracy measures
Fragasso, A., Mannarella, C., Ciancio, A. et al. (2012) Holotranscobalamin is a useful marker of vitamin B12 deficiency in alcoholics. Thescientificworldjournal 2012: 128182	- No diagnostic accuracy measures
Frenkel, E. P.; McCall, M. S.; White, J. D. (1971) An isotopic measurement of vitamin B12 in cerebrospinal fluid. American Journal of Clinical Pathology 55(1): 58-64	- No diagnostic accuracy measures
Friedner, S.; Josephson, B.; Levin, K. (1969) Vitamin B12 determination by means of radioisotope dilution and ultrafiltration. Clinica Chimica Acta 24(1): 171-9	- Full text paper not available
Girdwood, R. H. (1960) Microbiological methods of assay in clinical medicine with particular reference to the investigation of deficiency of vitamin B12 and folic acid. Scottish Medical Journal 5: 10-22	- No diagnostic accuracy measures
Gompertz, D. (1968) The measurement of urinary methylmalonic acid by a combination of thin-layer and gas chromoatography. Clinica Chimica Acta 19(3): 477-84	- No diagnostic accuracy measures
Hall, C. A. and Chu, R. C. (1990) Serum homocysteine in routine evaluation of potential vitamin B12 and folate deficiency. European Journal of Haematology 45(3): 143-9	- No diagnostic accuracy measures
Hannibal, L., Lysne, V., Bjorke-Monsen, A. L. et al. (2017) Corrigendum: Biomarkers and Algorithms for the Diagnosis of Vitamin B12	- Correction only

Study	Code [Reason]
Deficiency. Frontiers in Molecular Biosciences 4: 53	
Herbert, V., Colman, N., Palat, D. et al. (1984) Is there a "gold standard" for human serum vitamin B12 assay?. Journal of Laboratory & Clinical Medicine 104(5): 829-41	- Study design not relevant to this review protocol
Herrmann, W., Obeid, R., Schorr, H. et al. (2005) The usefulness of holotranscobalamin in predicting vitamin B12 status in different clinical settings. Current Drug Metabolism 6(1): 47-53	- No diagnostic accuracy measures
Herrmann, W., Obeid, R., Schorr, H. et al. (2003) Functional vitamin B12 deficiency and determination of holotranscobalamin in populations at risk. Clinical Chemistry & Laboratory Medicine 41(11): 1478-88	- Full text paper not available
Herrmann, W., Schorr, H., Bodis, M. et al. (2000) Role of homocysteine, cystathionine and methylmalonic acid measurement for diagnosis of vitamin deficiency in high-aged subjects. European Journal of Clinical Investigation 30(12): 1083-9	- No diagnostic accuracy measures
Herzlich, B. and Herbert, V. (1988) Depletion of serum holotranscobalamin II. An early sign of negative vitamin B12 balance. Laboratory Investigation 58(3): 332-7	- Population not relevant to this review protocol
Ho, C. H.; Chang, H. C.; Yeh, S. F. (1987) Quantitation of urinary methylmalonic acid by gas chromatography mass spectrometry and its clinical applications. European Journal of Haematology 38(1): 80-4	- No diagnostic accuracy measures
Honzik, T., Adamovicova, M., Smolka, V. et al. (2010) Clinical presentation and metabolic consequences in 40 breastfed infants with nutritional vitamin B12 deficiency--what have we learned?. European Journal of Paediatric Neurology 14(6): 488-95	- Population not relevant to this review protocol
Hvas, A. M., Ellegaard, J., Lous, J. et al. (2003) Health technology assessment in clinical biochemistry. Methylmalonic acid: a Danish showcase. Scandinavian Journal of Clinical & Laboratory Investigation 63(5): 319-30	- No diagnostic accuracy measures
Hvas, A. M., Lous, J., Ellegaard, J. et al. (2002) Use of plasma methylmalonic acid in diagnosing vitamin B-12 deficiency in general practice. Scandinavian Journal of Primary Health Care 20(1): 57-9	- No reference standard
Hvas, A. M. and Nexo, E. (2005) Holotranscobalamin--a first choice assay for diagnosing early vitamin B deficiency?. Journal of internal medicine 257(3): 289-298	- No diagnostic accuracy measures
Hvas, A. M. and Nexo, E. (2003) Holotranscobalamin as a predictor of vitamin B12 status. Clinical Chemistry & Laboratory Medicine 41(11): 1489-92	- Reference standard not measured in all participants
Iqbal, N., Azar, D., Yun, Y. M. et al. (2013) Serum methylmalonic acid and	- No diagnostic accuracy measures

Study	Code [Reason]
holotranscobalamin-II as markers for vitamin B12 deficiency in end-stage renal disease patients. Annals of Clinical & Laboratory Science 43(3): 243-9	
Jacobs, W. L. and Zondag, H. A. (1969) Radioisotope assay of vitamin B12 in human blood serum. Clinica Chimica Acta 24(1): 93-103	- Full text paper not available
Johannsen, P., Ostergaard, K., Christensen, J. E. et al. (1995) Methylmalonic acid in serum from patients with neurological symptoms consistent with cobalamin deficiency. European Journal of Neurology 2(4): 357-62	- Reference standard not measured in all participants
Joosten, E., Pelemans, W., Devos, P. et al. (1993) Cobalamin absorption and serum homocysteine and methylmalonic acid in elderly subjects with low serum cobalamin. European Journal of Haematology 51(1): 25-30	- Study design not relevant to this review protocol
Kalay, Z., Islek, A., Parlak, M. et al. (2016) Reliable and powerful laboratory markers of cobalamin deficiency in the newborn: plasma and urinary methylmalonic acid. Journal of Maternal-Fetal & Neonatal Medicine 29(1): 60-3	- Population not relevant to this review protocol
Kankra, M., Manocha, A., Bhargava, S. et al. (2015) Holotranscobalamin (HoloTC) an optimal and early marker of vitamin B12 deficiency and changes in cobalamin homeostasis-a silent epidemic with serious consequences. Indian Journal of Clinical Biochemistry 30(suppl1): 96	- Conference abstract
Kaushal, K. (2015) Holotranscobalamin and MethylMalonic Acid as the Diagnostic Tool for Vitamin B12 Deficiency. Indian Journal of Dermatology 60(6): 620	- Not a peer-reviewed publication
Killander, A. (1957) The use of the serum vitamin B12 assay in the diagnosis of vitamin B12 deficiency. Acta Medica Scandinavica 159(4): 307-21	- No diagnostic accuracy measures
Kumar, S.; Ghosh, K.; Das, K. C. (1989) Serum vitamin B12 levels in an Indian population: an evaluation of three assay methods. Medical Laboratory Sciences 46(2): 120-6	- Study design not relevant to this review protocol
Kwok, T., Cheng, G., Lai, W. K. et al. (2004) Use of fasting urinary methylmalonic acid to screen for metabolic vitamin B12 deficiency in older persons. Nutrition 20(9): 764-8	- Population not relevant to this review protocol
Lawrence, C. (1966) The binding of vitamin B12 by serum proteins in normal and B12-deficient subjects. British Journal of Haematology 12(5): 569-77	- Study design not relevant to this review protocol
Lee, S. M., Oh, J., Chun, M. R. et al. (2019) Methylmalonic Acid and Homocysteine as Indicators of Vitamin B12 Deficiency in Patients with Gastric Cancer after Gastrectomy. Nutrients 11(2): 21	- No diagnostic accuracy measures
Lee, Y. K.; Kim, H. S.; Kang, H. J. (2009) Holotranscobalamin as an indicator of vitamin B12 deficiency in gastrectomized patients.	- No diagnostic accuracy measures

Study	Code [Reason]
Annals of Clinical & Laboratory Science 39(4): 361-6	
Lindenbaum, J., Savage, D. G., Stabler, S. P. et al. (1990) Diagnosis of cobalamin deficiency: II. Relative sensitivities of serum cobalamin, methylmalonic acid, and total homocysteine concentrations. American Journal of Hematology 34(2): 99-107	- Study design not relevant to this review protocol
Lindgren, A.; Lindstedt, G.; Kilander, A. F. (1998) Advantages of serum pepsinogen A combined with gastrin or pepsinogen C as first-line analytes in the evaluation of suspected cobalamin deficiency: a study in patients previously not subjected to gastrointestinal surgery. Journal of Internal Medicine 244(4): 341-9	- Study does not contain any relevant index tests
Lindgren, A., Swolin, B., Nilsson, O. et al. (1997) Serum methylmalonic acid and total homocysteine in patients with suspected cobalamin deficiency: a clinical study based on gastrointestinal histopathological findings. American Journal of Hematology 56(4): 230-8	- Study aiming to diagnose malabsorption, not B12 deficiency
Linnell, J. C. (1981) The value of radioisotopic assays for "serum B12" in the diagnosis of cobalamin deficiency disorders. Clinical & Laboratory Haematology 3(2): 99-106	- Review article but not a systematic review
Lloyd-Wright, Z., Hvas, A. M., Moller, J. et al. (2003) Holotranscobalamin as an indicator of dietary vitamin B12 deficiency. Clinical Chemistry 49(12): 2076-8	- No diagnostic accuracy measures
Loikas, S., Lopponen, M., Suominen, P. et al. (2003) RIA for serum holo-transcobalamin: method evaluation in the clinical laboratory and reference interval. Clinical Chemistry 49(3): 455-62	- No diagnostic accuracy measures
Matchar, D. B. and Feussner, J. R. (1986) Laboratory tests in the diagnosis of vitamin B12 (cobalamin) deficiency. North Carolina Medical Journal 47(3): 118-20	- Review article but not a systematic review
McMullin, M. F., Young, P. B., Bailie, K. E. et al. (2001) Homocysteine and methylmalonic acid as indicators of folate and vitamin B12 deficiency in pregnancy. Clinical & Laboratory Haematology 23(3): 161-5	- No diagnostic accuracy measures
Merrigan, S. D.; Owen, W. E.; Straseski, J. A. (2015) Performance characteristics of the ARCHITECT Active-B12 (Holotranscobalamin) assay. Clinical Laboratory 61(34): 283-8	- Study design not relevant to this review protocol
Miller, A., Slingerland, D. W., Hall, C. A. et al. (1998) Food-bound B12 absorption and serum total homocysteine in patients with low serum B12 levels. American Journal of Hematology 59(1): 42-5	- Study aiming to diagnose malabsorption, not B12 deficiency
Miller, J. W., Garrod, M. G., Rockwood, A. L. et al. (2006) Measurement of total vitamin B12 and holotranscobalamin, singly and in combination,	- Population not relevant to this review protocol

Study	Code [Reason]
in screening for metabolic vitamin B12 deficiency. Clinical Chemistry 52(2): 278-85	
Moelby, L., Nielsen, G., Rasmussen, K. et al. (1997) Metabolic cobalamin deficiency in patients with low to low-normal plasma cobalamins. Scandinavian Journal of Clinical & Laboratory Investigation 57(3): 209-15	- No diagnostic accuracy measures
Moridani, M. and Ben-Poorat, S. (2006) Laboratory investigation of vitamin B12 deficiency. Laboratory Medicine 37(3): 166-174	- Review article but not a systematic review
Murphy, M. J., Brandie, F., Ebare, M. et al. (2021) Personalising laboratory medicine in the 'real world': Assessing clinical utility, by clinical indication, of serum total B12 and Active-B12 R (holotranscobalamin) in the diagnosis of vitamin B12 deficiency. Annals of Clinical Biochemistry 58(5): 445-451	- No diagnostic accuracy measures
Nexo, E., Christensen, A. L., Hvas, A. M. et al. (2002) Quantification of holo-transcobalamin, a marker of vitamin B12 deficiency. Clinical Chemistry 48(3): 561-2	- No diagnostic accuracy measures
Nilsson, K.; Gustafson, L.; Hultberg, B. (1999) Plasma homocysteine is a sensitive marker for tissue deficiency of both cobalamines and folates in a psychogeriatric population. Dementia & Geriatric Cognitive Disorders 10(6): 476-82	- No diagnostic accuracy measures
Nilsson, K., Isaksson, A., Gustafson, L. et al. (2004) Clinical utility of serum holotranscobalamin as a marker of cobalamin status in elderly patients with neuropsychiatric symptoms. Clinical Chemistry & Laboratory Medicine 42(6): 637-43	- Population not relevant to this review protocol
Norman, E. J.; Martelo, O. J.; Denton, M. D. (1982) Cobalamin (vitamin B12) deficiency detection by urinary methylmalonic acid quantitation. Blood 59(6): 1128-31	- No diagnostic accuracy measures
Norman, E. J. and Morrison, J. A. (1993) Screening elderly populations for cobalamin (vitamin B12) deficiency using the urinary methylmalonic acid assay by gas chromatography mass spectrometry. American Journal of Medicine 94(6): 589-94	- Population not relevant to this review protocol
Obeid, R. and Herrmann, W. (2007) Holotranscobalamin in laboratory diagnosis of cobalamin deficiency compared to total cobalamin and methylmalonic acid. Clinical Chemistry & Laboratory Medicine 45(12): 1746-50	- Data not reported in an extractable format or a format that can be analysed
Ok Bozkaya, I., Yarali, N., Kizilgun, M. et al. (2017) Relationship Between the Levels of Holotranscobalamin and Vitamin B12 in Children. Indian Journal of Hematology & Blood Transfusion 33(4): 537-540	- Population not relevant to this review protocol
Palacios, G., Sola, R., Barrios, L. et al. (2013) Algorithm for the early diagnosis of vitamin B12	- Population not relevant to this review protocol

Study	Code [Reason]
deficiency in elderly people. Nutricion Hospitalaria 28(5): 1447-52	
Pierce, H. I. and Hillman, R. S. (1974) The value of the serum vitamin B12 level in diagnosing B12 deficiency. Blood 43(6): 915-21	- Comparator in study does not match that specified in this review protocol
Pott, J. W. R. (2014) Detection of vitamin B12 deficiency in alcohol abuse. Acta Ophthalmologica 92(1): e76-e77	- Commentary article
Pusparini, Alvina, Merijanti, L. T. et al. (2020) Cobalamin and methylmalonic acid as biomarkers of vitamin B12 deficiency in elderly. International Journal of Pharmaceutical Research 12(4): 2724-2730	- Full text paper not available
Rasmussen, K.; Moelby, L.; Jensen, M. K. (1989) Studies on methylmalonic acid in humans. II. Relationship between concentrations in serum and urinary excretion, and the correlation between serum cobalamin and accumulation of methylmalonic acid. Clinical Chemistry 35(12): 2277-80	- Reference standard not measured in all participants
Rasmussen, K., Vyberg, B., Pedersen, K. O. et al. (1990) Methylmalonic acid in renal insufficiency: evidence of accumulation and implications for diagnosis of cobalamin deficiency. Clinical Chemistry 36(8pt1): 1523-4	- Letter to editor
Raven, J. L., Robson, M. B., Morgan, J. O. et al. (1972) Comparison of three methods for measuring vitamin B 12 in serum: radioisotopic, euglena gracilis and Lactobacillus leichmannii. British Journal of Haematology 22(1): 21-31	- Study design not relevant to this review protocol
Refsum, H., Yajnik, C. S., Gadkari, M. et al. (2001) Hyperhomocysteinemia and elevated methylmalonic acid indicate a high prevalence of cobalamin deficiency in Asian Indians. American Journal of Clinical Nutrition 74(2): 233-41	- No diagnostic accuracy measures
Regland, B., Abrahamsson, L., Gottfries, C. G. et al. (1990) Vitamin B12 analogues, homocysteine, methylmalonic acid, and transcobalamins in the study of vitamin B12 deficiency in primary degenerative dementia. Dementia 1(5): 272-277	- No reference standard
Remacha, A. F., Sarda, M. P., Canals, C. et al. (2013) Combined cobalamin and iron deficiency anemia: a diagnostic approach using a model based on age and homocysteine assessment. Annals of Hematology 92(4): 527-31	- Population not relevant to this review protocol
Remacha, A. F., Sarda, M. P., Canals, C. et al. (2014) Role of serum holotranscobalamin (holoTC) in the diagnosis of patients with low serum cobalamin. Comparison with methylmalonic acid and homocysteine. Annals of Hematology 93(4): 565-9	- No diagnostic accuracy measures
Richterman, A., Vaidya, A., Brown, J. M. et al. (2018) In the Balance. New England Journal of Medicine 378(3): e5	- Not a peer-reviewed publication

Study	Code [Reason]
Rogers, L. M., Boy, E., Miller, J. W. et al. (2003) High prevalence of cobalamin deficiency in Guatemalan schoolchildren: associations with low plasma holotranscobalamin II and elevated serum methylmalonic acid and plasma homocysteine concentrations. American Journal of Clinical Nutrition 77(2): 433-40	- No diagnostic accuracy measures
Rothen, J. P., Walter, P. N., Tsakiris, D. A. et al. (2021) Identification of Patients with Cobalamin Deficiency Crucially Depends on the Diagnostic Strategy. Clinical Laboratory 67(5): 01	- No diagnostic accuracy measures
Rozmaric, T., Mitulovic, G., Konstantopoulou, V. et al. (2020) Elevated Homocysteine after Elevated Propionylcarnitine or Low Methionine in Newborn Screening Is Highly Predictive for Low Vitamin B12 and Holo-Transcobalamin Levels in Newborns. Diagnostics 10(9): 24	- Study does not contain an intervention relevant to this review protocol
Rudobielska, M., Kaczmarski, M., Grutowicz, A. et al. (1972) The serum level of vitamin B 12 in healthy and diseased children. Helvetica Paediatrica Acta 27(6): 617-23	- Study design not relevant to this review protocol
Sarafoglou, K., Rodgers, J., Hietala, A. et al. (2011) Expanded newborn screening for detection of vitamin B12 deficiency. JAMA 305(12): 1198-200	- Comparator in study does not match that specified in this review protocol
Savage, D. G., Lindenbaum, J., Stabler, S. P. et al. (1994) Sensitivity of serum methylmalonic acid and total homocysteine determinations for diagnosing cobalamin and folate deficiencies. American Journal of Medicine 96(3): 239-46	- No diagnostic accuracy measures
Scarpa, E., Candiotto, L., Sartori, R. et al. (2013) Undetected vitamin B12 deficiency due to false normal assay results. Blood Transfusion 11(4): 627-629	- Study design not relevant to this review protocol
Schneede, J., Dagnelie, P. C., Van Staveren, W. A. et al. (1994) Methylmalonic acid and homocysteine in plasma as indicators of functional cobalamin deficiency in infants on macrobiotic diets. Pediatric Research 36(2): 194-201	- Study design not relevant to this review protocol
Schroder, T. H., Mattman, A., Sinclair, G. et al. (2016) Reference interval of methylmalonic acid concentrations in dried blood spots of healthy, term newborns to facilitate neonatal screening of vitamin B12 deficiency. Clinical Biochemistry 49(1314): 973-8	- Population not relevant to this review protocol
Schroder, T. H.; Quay, T. A.; Lamers, Y. (2014) Methylmalonic acid quantified in dried blood spots provides a precise, valid, and stable measure of functional vitamin B-12 status in healthy women. Journal of Nutrition 144(10): 1658-63	- Study design not relevant to this review protocol
Schwarz, J., Morstadt, E., Dura, A. et al. (2015) Biochemical Identification of Vitamin B12 Deficiency in a Medical Office. Clinical Laboratory 61(7): 687-92	- Population not relevant to this review protocol

Study	Code [Reason]
Serefhanoglu, S., Aydogdu, I., Kekilli, E. et al. (2008) Measuring holotranscobalamin II, an early indicator of negative vitamin B12 balance, by radioimmunoassay in patients with ischemic cerebrovascular disease. Annals of Hematology 87(5): 391-5	- Population not relevant to this review protocol
Sheridan, B. L. and Pearce, L. C. (1985) Vitamin B12 assays compared by use of patients' sera with low vitamin B12 content. Clinical Chemistry 31(5): 734-6	- Study design not relevant to this review protocol
Shum, H. Y.; Streeter, A. M.; O'Neill, B. J. (1970) A modified isotopic dilution method for measuring the serum vitamin B 12 level. Medical Journal of Australia 1(23): 1144-7	- Population not relevant to this review protocol
Simonson, W. (2018) Vitamin B12 deficiency - detection and treatment considerations. Geriatric Nursing 39(4): 477-478	- Commentary article
Sobczynska-Malefora, A., Gorska, R., Pelisser, M. et al. (2014) An audit of holotranscobalamin ("Active" B12) and methylmalonic acid assays for the assessment of vitamin B12 status: application in a mixed patient population. Clinical Biochemistry 47(12): 82-6	- No diagnostic accuracy measures
Solomon, L. R. (2005) Cobalamin-responsive disorders in the ambulatory care setting: Unreliability of cobalamin, methylmalonic acid, and homocysteine testing. Blood 105(3): 978-985	- Study design not relevant to this review protocol
Stabler, S. P. (2000) Using homocysteine and related metabolites to diagnose vitamin deficiency states. Biofactors 11(12): 51-2	- Abstract only
Stabler, S. P., Allen, R. H., Savage, D. G. et al. (1990) Clinical spectrum and diagnosis of cobalamin deficiency. Blood 76(5): 871-81	- Study design not relevant to this review protocol
Stabler, S. P.; Lindenbaum, J.; Allen, R. H. (1996) The use of homocysteine and other metabolites in the specific diagnosis of vitamin B-12 deficiency. Journal of Nutrition 126(4suppl): 1266S-72S	- No diagnostic accuracy measures
Sukumar, N. and Saravanan, P. (2019) Investigating vitamin B12 deficiency. BMJ 365: l1865	- Study design not relevant to this review protocol
Totoskovic, D.; Dopsaj, V.; Martinovic, J. (2016) Methylmalonic acid and neutrophil morphometric index in laboratory diagnosis of cobalamin deficiency without macrocytosis. International Journal of Laboratory Hematology 38(3): 265-72	- Study does not contain any relevant index tests
Tripathi, S., Chourey, N., Hiremath, R.N. et al. (2022) Reassessing the role of homocysteine and holotranscobalamin levels in diagnosing vitamin b12 deficiency anemia. Asian Journal of Pharmaceutical and Clinical Research 15(3): 99-103	- Study design not relevant to this review protocol
Valente, E., Scott, J. M., Ueland, P. M. et al. (2011) Diagnostic accuracy of holotranscobalamin, methylmalonic acid, serum	- Population not relevant to this review protocol

Study	Code [Reason]
cobalamin, and other indicators of tissue vitamin B12 status in the elderly. Clinical Chemistry 57(6): 856-63	
van Roon-Djordjevic, B. and Cerfontain-van, Staalen (1972) Urinary excretion of histidine metabolites as an indication for folic acid and vitamin B 12 deficiency. Clinica Chimica Acta 41: 55-65	- Full text paper not available
Vashi, P., Edwin, P., Popiel, B. et al. (2016) Methylmalonic Acid and Homocysteine as Indicators of Vitamin B-12 Deficiency in Cancer. PLoS ONE [Electronic Resource] 11(1): e0147843	- Population not relevant to this review protocol
Verma, A., Aggarwal, S., Garg, S. et al. (2020) Comparison of Serum Holotranscobalamin with Serum Vitamin B12 in People Prone to Megaloblastic Anemia and Correlation with Nerve Conduction Study. Journal of the Association of Physicians of India 68(1): 101	- Study design not relevant to this review protocol
Vugteveen, I., Hoeksma, M., Monsen, A. L. et al. (2011) Serum vitamin B12 concentrations within reference values do not exclude functional vitamin B12 deficiency in PKU patients of various ages. Molecular Genetics & Metabolism 102(1): 13-7	- No diagnostic accuracy measures
Waldenlind, L.; Lamminpaa, K.; Sundblad, L. (1982) Determination of 'total' and 'true' cobalamin with the simulTRAC Assay. Scandinavian Journal of Clinical and Laboratory Investigation 42(3): 225-229	- No reference standard
Warendorf, J. K., van Doormaal, P. T. C., Vrancken, Afje et al. (2021) Clinical relevance of testing for metabolic vitamin B12 deficiency in patients with polyneuropathy. Nutritional Neuroscience: 1-11	- Population not relevant to this review protocol
Webb, M. G.; Weir, D. G.; Moore, J. N. (1971) Diagnosis of vitamin B 12 deficiency in psychiatric patients. Journal of the Irish Medical Association 64(417): 403-8	- No diagnostic accuracy measures
Wickramasinghe, S. N. and Fida, S. (1993) Correlations between holo-transcobalamin II, holo-haptocorrin, and total B12 in serum samples from healthy subjects and patients. Journal of Clinical Pathology 46(6): 537-9	- Study design not relevant to this review protocol
Wide, L. and Killander, A. (1971) A radiosorbent technique for the assay of serum vitamin B12. Scandinavian Journal of Clinical & Laboratory Investigation 27(2): 151-9	- No diagnostic accuracy measures
Witherspoon, L. R. (1981) Vitamin B12: are serum radioassay measurements reliable?. Journal of Nuclear Medicine 22(5): 474-7	- Not a peer-reviewed publication
Woo, K. S., Kim, K. E., Park, J. S. et al. (2010) Relationship between the Levels of Holotranscobalamin and Vitamin B12. Korean Journal Of Laboratory Medicine 30(2): 185-9	- No diagnostic accuracy measures

Study	Code [Reason]
Yazdanpanah, M., Chan, P. C., Evrovski, J. et al. (2003) An improved assay for plasma methylmalonic acid using chemical ionization gas chromatography mass spectrometry. Clinical Biochemistry 36(8): 617-20	- No reference standard
Zhou, P., Hua, H., Yan, Z. et al. (2018) Diagnostic value of oral "beefy red" patch in vitamin B12 deficiency. Therapeutics & Clinical Risk Management 14: 1391-1397	- Population not relevant to this review protocol

Health Economic studies

Published health economic studies that met the inclusion criteria (relevant population, comparators, economic study design, published 2005 or later and not from non-OECD country or USA) but that were excluded following appraisal of applicability and methodological quality are listed below. See the health economic protocol for more details.

Table 25: Studies excluded from the health economic review

Reference	Reason for exclusion
None	-

J.2 Intervention

Clinical studies

No excluded studies

Health Economic studies

No excluded studies

Appendix K – Research recommendations – full details

K.1 Research recommendation

What are the long-term outcomes for people with suspected vitamin B12 deficiency when comparing testing of total serum B12 (serum cobalamin), active B12 (holotranscobalamin), methylmalonic acid (MMA) or homocysteine?

K.1.1 Why this is important

The concentration of cobalamin (serum B12 test), holotranscobalamin (also known as 'active B12'), methylmalonic acid (MMA) and total homocysteine in the blood can all be used to diagnose vitamin B12 deficiency, although the accuracy of each biomarker may vary between different patient groups. There is no single, widely adopted diagnostic algorithm for the diagnosis of vitamin B12 deficiency. No evidence was identified to determine which test or combination of tests leads to the best outcomes for people with suspected vitamin B12 deficiency. More evidence is needed on the long-term outcomes of different testing strategies, particularly for quality of life and patient reported outcomes.

K.1.2 Rationale for research recommendation

Importance to 'patients' or the population	More accurate diagnostic tests facilitate faster and more accurate diagnoses, leading to better outcomes for patients. By comparing the long-term outcomes of patients undergoing different tests for vitamin B12 deficiency, the most clinically effective test can be established and recommended in future guideline updates.
Relevance to NICE guidance	High: the research is essential to inform future updates of key recommendations in the guidance.
Relevance to the NHS	The outcome would affect the type of test being offered by the NHS for diagnosis of vitamin B12 deficiency.
National priorities	Getting It Right First Time (GIRFT) has started work looking at the approaches taken to identify B12 status and reduce 'waste' in the system.
Current evidence base	There is no randomised trial data on the effectiveness of the tests.
Equality considerations	The research recommendation addresses equality issues related to age, pregnancy, ethnicity, and sex. Reference ranges differ in these groups, so test results based on manufacturer cut-offs may not be appropriate. It is therefore recommended that outcomes in these groups are studied separately.

K.1.3 Modified PICO table

Population	People with suspected vitamin B12 deficiency. Stratified by: • Age (children below 16/18 years; adults 16/18 years and older; older adults 65 years and older)

	• Third trimester of pregnancy (third trimester; first two trimesters and not pregnant) • Ethnicity (Afro-Caribbean; other) • Sex (male; female) for Homocysteine test only
Interventions	The following as stand-alone tests or in combination: • Serum cobalamin assay • Holotranscobalamin test • Methylmalonic acid test (including urinary) • Homocysteine test Followed by vitamin B12 replacement as a result of a positive test. Strata: reference range
Comparator	Each other

Outcomes	• quality of life (such as EQ5D, SF36)
	• patient-reported outcomes (PROM scores including some/all symptoms):
	  ○ fatigue
	  ○ sleep
	  ○ peripheral neuropathy
	  ○ cognition
	  ○ psychiatric symptoms
	  ○ pain
	• haematological values
	• complications and adverse events
	  ○ mortality
	  ○ bleeds
	  ○ self-harm
	  ○ nerve damage
	  ○ frailty/falls
	  ○ severe cognitive effects
	  ○ postural hypotension
	• patient concern around unexpected lab results (health anxiety score)
	• incorrect/delayed diagnosis
	• inappropriate additional tests
	• adherence to treatment
	• school/education/work absence

Study design	Randomised controlled trial
Timeframe	Long term (12 months)
Additional information	None

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