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Federal Department of Home Affairs
Federal Office of Public Health FOPH
Health and Accident Insurance Directorate
Section Health Technology Assessment

Health Technology Assessment (HTA)

HTA Short Report

Title	Effectiveness and safety of vitamin B12 tests
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Technology	Vitamin B12 testing in the general population and in high-risk populations. Analysis list («Analysenliste») Pos. Nr. 1749.00; TP: 25: Vitamin B12, Cyanocobalamin Pos. Nr. 1568.00; TP: 110: Methylmalonic acid (MMA) (Limitation: second tier test for vitamin B12 deficiency) Pos. Nr. 1727.10; TP: 61: Holotranscobalamin (holoTC)
Date	11 June 2021
Type of Technology	Laboratory analyses

Conflicts of Interest:

The authors have no financial, academic, personal or any other conflicts of interest to declare in relation to this project.

Executive Summary

The objective of this health technology assessment (HTA) short report was to evaluate the benefits and harms associated with vitamin B12 tests (i.e. serum B12, holotranscobalamin [holoTC] and methylmalonic acid [MMA]) in patients with a clinical suspicion of, or at high risk for, vitamin B12 deficiency. In addition, legal, social, ethical and organisational issues associated with vitamin B12 testing were considered. The applicability of such findings has been discussed within the Swiss context.

Clinical evaluation

Systematic literature searches were conducted to identify existing systematic review (SR) evidence addressing the research questions. In addition, primary literature was sought to fill gaps in existing SR evidence, and to update existing SRs where appropriate. Direct evidence evaluating the impact of B12 testing on patient-relevant outcomes from randomised or non-randomised trials was sought with priority. In the absence of direct evidence, a linked evidence approach was undertaken. *De novo* meta-analysis was outside the scope of this evaluation.

Direct evidence

No direct evidence evaluating the impact of B12 testing on patient-relevant outcomes was identified.

Linked evidence: test accuracy

One existing SR evaluating the diagnostic accuracy of serum B12 testing (searches up to November 2009) was identified (k = 36). No existing reviews of holoTC or MMA were identified. In addition, six primary studies published since November 2009 were included. Of these, six evaluated vitamin B12 (n = 15,608), five evaluated holoTC (n = 15,531), and two evaluated MMA (n = 11,910). All primary studies were considered to be at risk of bias.

Sensitivity of the vitamin B12 test against MMA as a reference standard ranged from 52% to 72%, reported by one SR and three primary diagnostic accuracy studies. Specificity ranged from 41% to 81%. A primary study conducted in Switzerland with a large sample size and novel reference standard (4cB12 method) reported sensitivity of 86% and specificity of 78% in identifying patients with subclinical vitamin B12 deficiency. The quality of evidence was very low.

Two paired single-gate diagnostic accuracy studies (n = 2,638) and one paired two-gate diagnostic accuracy study (n = 360) that investigated holoTC as an index test and used MMA as a reference standard reported sensitivity ranging from 56% to 83%, and specificity from 51%

to 60%. The paired two-gate diagnostic accuracy study that employed the 4cB12 model as a reference standard reported sensitivity and specificity of 86% and 81%, respectively, in possibly deficient cases (n = 51). The quality of evidence was low.

One paired single-gate diagnostic accuracy study used MMA as an index test, one used holoTC as a reference standard (n = 77), and one used a 4cB12 model (n = 11,833). The holoTC study reported sensitivity and specificity of 40% and 94%, respectively (low quality). The 4cB12 study reported sensitivity and specificity of 82% and 83%, respectively (low quality). Serum B12 measured with holoTC as the reference standard typically yielded lower sensitivity and specificity compared to studies that used 4cB12 as the reference standard. The quality of evidence was low.

Linked evidence: change in management

Three cross-sectional studies evaluated the impact of MMA and B12 testing on clinical management decisions (n = 608). No studies regarding holoTC testing were identified. Two of these studies concluded that hospital patients who received test results indicating low levels of vitamin B12 or MMA were not managed according to recommendations. One study reported that MMA was followed up appropriately in Danish GP clinics, however, it was often used as a screening test in asymptomatic patients. The studies were at a high risk of bias.

Linked evidence: effectiveness of change in management

Eight SRs reviewed vitamin B12 supplementation in patients with a B12 deficiency. The studies reviewed populations with vitamin B12 deficiency related to a gastrointestinal disorder, pernicious anaemia, before and/or after bariatric surgery, neurodegenerative disorder and/or cognitive impairment, or of unknown aetiology. These studies investigated differences between the dosage and route of vitamin B12 supplementation. In participants with deficient vitamin B12 levels, high doses (i.e. 1,000 µg) of orally administered vitamin B12 was found to be as effective as intramuscular administration in correcting vitamin B12 deficiency and achieving clinical responses. The reviews did not evaluate the safety of supplementation. The risk of bias in the included reviews ranged from low to high.

Five SRs and 30 primary research studies investigated the natural course of vitamin B12 deficiency. These studies reported that insufficient vitamin B12 levels, when left untreated, can lead to conditions ranging from mild to severe (e.g., neurological abnormalities, cognitive impairment, haematological abnormalities, complications during pregnancy).

Social, legal, ethical and organisational issues

Systematic and non-systematic searches identified 22 relevant studies of primary and secondary research. Social and organisational issues related to B12 testing included the medicalisation of vitamins and the subsequent rise in unnecessary testing. Physicians recommended several way to reduce unnecessary vitamin B12 testing in practice: additional educational resources and contemporary guidelines, longer consultation times, and removal of vitamin B12 tests from testing kits. A key ethical concern was related to false positives and false negatives; however, the potential harm caused by these erroneous outcomes is minimised because it takes several years for vitamin B12 levels to become depleted, vitamin deficiency is treatable through supplementation, and physicians are likely to prescribe supplementation for borderline cases of vitamin B12 deficiency. No legal issues were identified.

Conclusion

There is currently no evidence directly measuring the impact of B12 tests on patient reported outcomes. Similarly, there do not appear to be widely accepted clinical standards for the indications, number and order of tests, and diagnostic thresholds for B12 testing in Swiss practice. Evaluating the diagnostic accuracy of B12 tests is complicated by the absence of an established reference standard, the presence of confounding factors (e.g. renal insufficiency, age), and variable reference thresholds for diagnosis. The current evidence does not inform the most appropriate test, or combination of tests to investigate a patient with suspected, or at high risk of, B12 deficiency, nor the utility of such tests on impacting patient-relevant outcomes.

Zusammenfassung

Ziel dieses Kurzberichts zum Health Technology Assessment (HTA) war es, die Vor- und Nachteile im Zusammenhang mit Vitamin-B12-Tests (d. h. Serum B12, Holotranscobalamin [Holo-TC] und Methylmalonsäure [MMS]) bei Patientinnen und Patienten mit klinischem Verdacht auf oder hohem Risiko für Vitamin-B12-Mangel aufzuzeigen. Weiter wurden rechtliche, soziale, ethische und organisatorische Fragen bezüglich der Durchführung von Vitamin-B12-Tests betrachtet. Die Anwendbarkeit solcher Erkenntnisse wurde im schweizerischen Kontext diskutiert.

Klinische Evaluation

Mithilfe systematischer Literaturrecherchen wurde die Evidenz bestehender systematischer Reviews (SR) zu Forschungsfragen erhoben. Ausserdem wurde nach Primärliteratur gesucht, um Lücken in der bestehenden SR-Evidenz zu schliessen und bestehende SRs, bei Bedarf, zu aktualisieren. Es wurde prioritär nach direkter Evidenz zur Evaluation der Auswirkungen von

B12-Tests auf patientenrelevante Ergebnisse aus randomisierten oder nichtrandomisierten Studien gesucht. Da direkte Evidenz fehlte, wurde ein Linked-Evidence-Ansatz verfolgt. Die De-novo-Meta-Analyse gehörte nicht zum Fokus dieser Evaluation.

Direkte Evidenz

Es wurde keine direkte Evidenz für die Beurteilung der Auswirkungen von B12-Tests auf patientenrelevante Ergebnisse gefunden.

Linked Evidence: Testgenauigkeit

Es wurde eine bestehende SR identifiziert, die sich mit der Diagnosegenauigkeit von Serum-B12-Tests befasste (Suchvorgänge bis November 2009) (k = 36). Es wurden keine bestehenden Reviews für Holo-TC oder MMS gefunden. Zudem wurden sechs Primärstudien, die seit November 2009 veröffentlicht worden waren, eingeschlossen. Von diesen Studien befassten sich sechs mit Vitamin B12 (n = 15 608), fünf mit Holo-TC (n = 15 531) und zwei mit MMS (n = 11 910). Bei allen Primärstudien wurde ein Verzerrungspotenzial festgestellt.

Eine SR und drei Primärstudien zur Diagnosegenauigkeit ergaben eine Sensitivität von Vitamin-B12-Tests gegenüber MMS als Referenzstandard von 52 bis 72 Prozent. Die Spezifität variierte zwischen 41 und 81 Prozent. Eine in der Schweiz mit einer grossen Stichprobe und einem neuartigen Referenzstandard (4cB12-Methode) durchgeführte Primärstudie wies bei der Identifikation von Patientinnen und Patienten mit einem subklinischen Vitamin-B12-Mangel eine Sensitivität von 86 Prozent und eine Spezifität von 78 Prozent auf. Die Qualität der Evidenz war sehr gering.

Zwei gekoppelte Single-Gate-Studien zur Diagnosegenauigkeit (n = 2638) und eine gekoppelte Two-Gate-Studie zur Diagnosegenauigkeit (n = 360), die Holo-TC als Indextest untersuchten und MMS als Referenzstandard verwendeten, ergaben eine Sensitivität von 56 bis 83 Prozent und eine Spezifität zwischen 51 und 60 Prozent. Die gekoppelte Two-Gate-Studie zur Diagnosegenauigkeit, die das 4cB12-Modell als Referenzstandard anwandte, wies eine Sensitivität und eine Spezifität von 86 bzw. 81 Prozent auf, in möglicherweise unzureichenden Fällen (n = 51). Die Qualität der Evidenz war gering.

Eine gekoppelte Single-Gate-Studie zur Diagnosegenauigkeit verwendete MMS als Indextest, eine andere Holo-CT als Referenzstandard (n = 77) und eine weitere ein 4cB12-Modell (n = 11 833). Die Holo-TC-Studie ergab eine Sensitivität und eine Spezifität von 40 bzw. 94 Prozent (geringe Qualität). Die 4cB12-Studie wies eine Sensitivität und eine Spezifität von 82 bzw. 83 Prozent aus (geringe Qualität). Serum B12 gemessen mit Holo-TC als Referenzstandard ergab

typischerweise eine tiefere Sensitivität und Spezifität im Vergleich zu Studien, die 4cB12 als Referenzstandard nutzten. Die Qualität der Evidenz war gering.

Linked Evidence: ein anderes Management

Drei Querschnittstudien evaluierten die Auswirkungen von MMS- und B12-Tests auf Entscheidungen im klinischen Management (n = 608). Es wurden keine Studien in Bezug auf die Durchführung von Holo-TC-Tests gefunden. Zwei dieser Studien kamen zum Schluss, dass Spitalpatientinnen und -patienten, deren Testergebnisse geringe Vitamin-B12- oder MMA-Spiegel aufwiesen, nicht gemäss den Empfehlungen betreut wurden. Laut einer Studie wurde MMS in dänischen Hausarztpraxen angemessen verfolgt, kam jedoch häufig als Screeningtest bei asymptomatischen Patientinnen und Patienten zum Einsatz. Die Studien wiesen ein hohes Verzerrungspotenzial auf.

Linked Evidence: Effektivität eines anderen Managements

Acht SRs befassten sich mit der Vitamin-B12-Supplementierung bei Patientinnen und Patienten mit B12-Mangel. Die Studien betrachteten Bevölkerungsgruppen mit Vitamin-B12-Mangel im Zusammenhang mit einer Magen-Darm-Erkrankung, einer perniziösen Anämie, vor und/oder nach einem bariatrischen Eingriff, mit einer neurodegenerativen Erkrankung und/oder einer kognitiven Beeinträchtigung oder einer unbekannten Ätiologie. Diese Studien untersuchten die Unterschiede bei der Dosierung und dem Verlauf der Vitamin-B12-Supplementierung. Es wurde festgestellt, dass im Hinblick auf die Behebung des Vitamin-B12-Mangels und die Erzielung klinischer Reaktionen bei Teilnehmenden mit unzureichenden Vitamin-B12-Spiegeln hochdosiertes oral verabreichtes Vitamin B12 (d. h. 1000 µg) ebenso effektiv war wie die intramuskuläre Verabreichung. Diese Reviews haben die Sicherheit der Supplementierung nicht evaluiert. Das Verzerrungspotenzial der berücksichtigten Reviews variierte von tief bis hoch.

Fünf SRs und 30 Primärforschungsstudien untersuchten den natürlichen Verlauf von Vitamin-B12-Mangel. Diesen Studien zufolge können unzureichende Vitamin-B12-Spiegel unbehandelt zu leichten bis schweren Symptomen führen (z. B. zu neurologischen Anomalien, kognitiven Beeinträchtigungen, hämatologischen Anomalien, Komplikationen während der Schwangerschaft).

Soziale, rechtliche und organisatorische Fragen

Systematische und nichtsystematische Recherchen ergaben 22 relevante Studien aus der primären und der sekundären Forschung. Soziale und organisatorische Fragen im Zusammenhang mit der Durchführung von Vitamin-B12-Tests umfassten die Medikalisierung von Vitaminen und die daraus resultierende Zunahme unnötiger Tests. Ärztinnen und Ärzte

empfahlen verschiedene Möglichkeiten, um unnötige Vitamin-B12-Tests in der Praxis zu reduzieren: zusätzliche pädagogische Ressourcen und zeitgemässe Vorgaben, eine längere Konsultationsdauer und das Entfernen von Vitamin-B12-Tests aus Testkits. Ein wichtiges ethisches Anliegen betraf falsch-positive und falsch-negative Ergebnisse. Allerdings ist der potenzielle Schaden dieser falschen Ergebnisse äusserst gering, da es mehrere Jahre braucht, bis Vitamin-B12-Spiegel ein unzureichendes Niveau erreichen, da einem Vitaminmangel mit Supplementierung entgegengewirkt werden kann und da Ärzte bei Grenzfällen von Vitamin-B12-Mangel wahrscheinlich eine Supplementierung verschreiben. Es wurden keine rechtlichen Fragen festgestellt.

Fazit

Es gibt momentan keine Evidenz, die die Auswirkungen von B12-Tests auf von Patientinnen und Patienten berichtete Ergebnisse misst. Ebenso scheint es in der schweizerischen Praxis keine breit akzeptierten klinischen Standards für die Indikationen, für die Anzahl und die Reihenfolge von Tests sowie für die Diagnoseschwellen für B12-Tests zu geben. Die Evaluation der Diagnosegenauigkeit von B12-Tests gestaltet sich wegen des Fehlens eines etablierten Referenzstandards, des Vorhandenseins von Störfaktoren (z. B. Niereninsuffizienz, Alter) und variabler Referenzschwellen für die Diagnose kompliziert. Die vorhandene Evidenz gibt weder Aufschluss über den geeignetsten Test oder die beste Testkombination für die Untersuchung eines Patienten oder einer Patientin mit Verdacht auf oder hohem Risiko für Vitamin-B12-Mangel noch über den Nutzen solcher Tests für die Beeinflussung patientenrelevanter Ergebnisse.

Synthèse

La présente synthèse d'évaluation des technologies de la santé (ETS) visait à évaluer les avantages et les inconvénients des tests de vitamine B12 (p. ex. sérum B12, holotranscobalamine [holoTC] et acide méthyl-malonique [AMM]) en cas de suspicion clinique ou de risque élevé de carence en vitamine B12. En outre, les aspects juridiques, sociaux, éthiques et organisationnels associés aux tests de vitamine B12 ont été pris en considération. L'applicabilité de telles observations a fait l'objet de discussions dans le contexte de la Suisse.

Évaluation clinique

Des recherches bibliographiques ont permis de recenser, dans les re-vues systématiques (RS), les données scientifiques sur certains aspects de la recherche. De plus, la littérature primaire a été compilée en vue de compléter les données factuelles dans les RS existantes et, si nécessaire, de mettre à jour ces dernières. Les preuves directes ont été recherchées en priorité

pour évaluer l'impact des tests de vitamine B12 sur les résultats concernant les patients et issus d'essais randomisés ou non randomisés. En l'absence de preuves directes, il a été procédé à une approche fondée sur les preuves directes (linked evidence ap-proach, LSA). La méta-analyse de novo n'entrait pas dans le champ de la présente évaluation.

Preuves directes

Aucune preuve directe évaluant l'impact des tests de vitamine B12 sur les résultats pertinents pour les patients n'a été trouvée.

Preuves indirectes : exactitude des tests

L'existence d'une RS évaluant la précision diagnostique des tests de sérum B12 (recherches jusqu'en novembre 2009) a été recensée (k = 36). Aucune revue existante sur la holoTC ou l'AMM n'a été trouvée. De plus, six études primaires publiées depuis novembre 2009 ont été incluses : les six évaluaient la vitamine B12 (n = 15,608), cinq évaluaient la holoTC (n = 15,531) et deux évaluaient l'AMM (n = 11,910). Toutes les études primaires ont été considérées comme présentant un risque de biais.

La sensibilité des tests de vitamine B12 par rapport à l'AMM comme norme de référence se situait entre 52 % et 72 %, comme indiqué par une RS et trois études primaires sur la précision diagnostique. La spéci-ficité se situait quant à elle entre 41 % et 81 %. Une étude primaire menée en Suisse avec un vaste échantillonnage et une nouvelle norme de référence (méthode 4cB12) indiquait une sensibilité de 86 % et une spécificité de 78 % dans le dépistage de patients présentant une ca-rence subclinique en vitamine B12. La qualité des données scientifiques était très faible.

Deux études monocritères couplées sur la précision diagnostique (n = 2,638) et une étude bicritère couplée sur la précision diagnostique (n = 360), qui ont étudié la holoTC en tant que test d'indice et utilisé l'AMM comme norme de référence indiquaient une sensibilité entre 56 % et 83 % et une spécificité entre 51 % et 60 %. L'étude bicritère couplée sur la précision diagnostique qui a appliqué le modèle 4cB12 comme norme de référence indiquait une sensibilité et une spécificité respecti-vement de 86 % et 81 % des cas de carence possible (n = 51). La qua-lité des données scientifiques était faible.

Une étude monocritère couplée sur la précision diagnostique a utilisé l'AMM en tant que test d'indice, l'une a utilisé la holoTC comme norme de référence (n = 77) et l'autre un modèle 4cB12 (n = 11 833). L'étude qui a utilisé la holoTC indiquait une sensibilité et une spécificité de respectivement 40 % et 94 % (qualité faible). Celle qui a utilisé le modèle 4cB12 indiquait une sensibilité et une spécificité de respectivement 82 % et 83 % (qualité faible). Le sérum B12

mesuré avec de la holoTC comme norme de référence a fourni typiquement une sensibilité et une spécificité inférieures comparé aux études qui ont utilisé le modèle 4cB12 comme norme de référence. La qualité des données scienti-fiques était faible.

Preuves indirectes : changement de de gestion clinique

Trois études transversales ont évalué l'impact des tests d'AMM et de vitamine B12 sur les décisions de gestion clinique (n = 608). Aucune étude concernant des tests de holoTC n'a été trouvée. Deux de ces études concluaient que les patients hospitalisés qui avaient reçu des résultats de tests présentant des taux bas de vitamine B12 ou d'AMM n'avaient pas été traités selon les recommandations. Une étude indi-quait que les résultats de l'AMM avaient été suivis de manière appro-priée dans des cabinets de soins généraux danois, toutefois, ils avaient souvent été utilisés comme tests de dépistage des patients asymptoma-tiques. Les études présentaient un risque de biais élevé.

Preuves indirectes : efficacité pratique du changement de gestion clinique

Huit RS ont examiné la supplémentation de vitamine B12 à des patients présentant une carence. Les études ont examiné des populations pré-sentant une carence en vitamine B12 en rapport avec des troubles gas-tro-intestinaux, une anémie pernicieuse avant et/ou après une chirurgie bariatrique, un trouble neurodégénératif et/ou un handicap cognitif, ou une étiologie inconnue. Ces études ont analysé les différences qui exis-tent entre le dosage et le parcours de la supplémentation de vitamine B12. Chez les participants présentant un taux insuffisant de vitamine B12, il s'est avéré que des doses élevées (à savoir 1,000 µg) de vita-mine B12 administrées oralement étaient aussi efficaces qu'une admi-nistration intramusculaire pour corriger la carence en vitamine B12 et obtenir des réponses cliniques. Les revues n'ont pas évalué la sécurité de la supplémentation. Le risque de biais des revues prises en compte était de bas à élevé.

Cinq RS et 30 études de recherche primaire ont analysé le parcours naturel de la carence en vitamine B12. Ces études indiquaient qu'un taux insuffisant de vitamine B12, si non traité, est susceptible d'induire des conditions cliniques modérées à sévères (p. ex. anomalies neurologiques, problèmes cognitifs, anomalies hématologiques, complications pendant la grossesse).

Aspects sociaux, juridiques, éthiques et organisationnels

Des recherches systématiques et non systématiques ont identifié 22 études pertinentes de recherche primaire et secondaire. Les aspects sociaux et organisationnels relatifs aux tests de vitamine B12 compre-naient la médicalisation des vitamines et l'augmentation subséquente de tests non nécessaires. Les praticiens recommandaient différents moyens pour réduire les tests

de vitamine B12 non nécessaires dans la pratique : un supplément de ressources didactiques et des directives mises à jour, des durées de consultation plus longues et la suppression des tests de vitamine B12 présents dans les kits de test. Une préoccu-pation éthique majeure était liée aux faux positifs et aux faux négatifs ; toutefois, le potentiel inconvénient causé par ces résultats erronés est minimisé, étant donné qu'il faut plusieurs années pour que les taux de vitamine B12 diminuent, que la carence en vitamine peut être traitée par une supplémentation et que les praticiens ont tendance à prescrire une supplémentation pour les cas problématiques de carence en vitamine B12. Aucun aspect juridique n'a été trouvé.

Conclusion

Il n'existe actuellement aucune donnée scientifique qui mesure directe-ment l'impact des tests de vitamine B12 sur les résultats déclarés par les patients. Similairement, il ne semble pas exister de normes cliniques largement acceptées pour les indications, le nombre et l'ordre des tests ni pour les seuils de diagnostic des tests de vitamine B12 dans la pra-tique suisse. L'évaluation de la précision diagnostique des tests de vi-tamine B12 est compliquée par l'absence de norme de référence éta-blie, la présence de facteurs aggravants (p. ex. insuffisance rénale, âge) et des seuils de référence variables pour le diagnostic. Les don-nées scientifiques actuelles ne fournissent aucune information quant au test le plus approprié ou à une combinaison de tests permettant d'examiner une suspicion ou un risque élevé de carence en vitamine B12, ni sur l'utilité de tels tests susceptibles d'avoir un impact sur les résultats concernant les patients.

Sintesi

L'obiettivo del presente rapporto breve di Health Technology Assess-ment (HTA) era quello di valutare i benefici e gli svantaggi associati ai test per la vitamina B12 (ovvero concentrazione sierica di vitamina B12, olotranscobalamina [oloTC] e acido metilmalonico [MMA]) nei pazienti che presentano un sospetto clinico o un elevato rischio di carenza di vitamina B12. Sono stati inoltre considerati aspetti di natura legale, so-ciale, etica e organizzativa associati a questa tipologia di test. L'applicabilità dei risultati ottenuti è stata poi discussa in seno al conte-sto svizzero.

Valutazione clinica

Sono state condotte ricerche bibliografiche sistematiche al fine di identi-ficare prove esistenti di revisione sistematica (RS) che affrontano le te-matiche oggetto di ricerca. Inoltre, sono state ricercate le fonti di biblio-grafia primaria per colmare le lacune nelle evidenze RS disponibili e, ove opportuno, per aggiornare le RS esistenti. La priorità nelle ricerche è stata attribuita alle

evidenze dirette che valutano l'impatto dei test per la vitamina B12 sui risultati rilevanti per il paziente tratti da studi randomiz-zati o non randomizzati. In assenza di un'evidenza diretta è stato adot-tato un approccio basato sulle evidenze indirette. La meta-analisi de novo si è collocata al di fuori del campo di applicazione di questa valuta-zione.

Evidenza diretta

Non è stata individuata alcuna evidenza diretta che valutasse l'impatto dei test per la vitamina B12 sui risultati rilevanti per i pazienti.

Evidenza indiretta: accuratezza dei test

È stata individuata una RS esistente che valuta l'accuratezza diagnosti-ca dei test per la concentrazione sierica di vitamina B12 (ricerche fino a novembre 2009) (k = 36). Non è stata individuata alcuna revisione esi-stente di oloTC o MMA. Sono stati inoltre inclusi sei studi primari pubbli-cati a partire da novembre 2009. Di questi, sei hanno valutato la vitami-na B12 (n = 15 608), cinque l'oloTC (n = 15 531) e due l'MMA (n = 11 910). Tutti gli studi primari sono stati considerati come a rischio di bias.

La sensibilità del test della vitamina B12 rispetto all'MMA come standard di riferimento si è collocata in un intervallo compreso tra il 52 % e il 72 %, come riportato da uno studio RS e da tre studi primari di accura-tezza diagnostica. La specificità si è collocata in un intervallo compreso tra il 41 % e l'81 %. Uno studio primario condotto in Svizzera con un campione di ampie dimensioni e un nuovo standard di riferimento (me-todo 4cB12) ha evidenziato una sensibilità dell'86 % e una specificità del 78 % nell'individuazione dei pazienti con una carenza subclinica di vita-mina B12. La qualità dell'evidenza è stata molto bassa.

Due studi accoppiati di accuratezza diagnostica a gruppo singolo (n = 2638) e uno studio accoppiato di accuratezza diagnostica a due gruppi (n = 360) che hanno esaminato l'impiego dell'oloTC come test indice e hanno utilizzato l'MMA come standard di riferimento hanno evidenziato una sensibilità compresa tra il 56 % e l'83 % e una specificità compresa tra il 51 % e il 60 %. Lo studio accoppiato di accuratezza diagnostica a due gruppi che ha impiegato il modello 4cB12 come standard di riferi-mento ha evidenziato una sensibilità e una specificità rispettivamente dell'86 % e dell'81 % in casi di possibile carenza (n = 51). La qualità dell'evidenza è stata bassa.

Uno studio accoppiato di accuratezza diagnostica a gruppo singolo ha utilizzato l'MMA come test indice, uno ha utilizzato l'oloTC come stan-dard di riferimento (n = 77) e uno ha utilizzato un modello 4cB12 (n = 11 833). Lo studio oloTC ha evidenziato una sensibilità e una specificità rispettivamente del 40 % e del 94 % (bassa qualità). Lo studio 4cB12 ha mostrato una sensibilità

e una specificità rispettivamente dell'82 % e dell'83 % (bassa qualità). La concentrazione sierica di vitamina B12 mi-surata con l'oloTC come standard di riferimento ha espresso tipicamen-te una sensibilità e una specificità inferiori rispetto agli studi che hanno utilizzato come standard di riferimento il modello 4cB12. La qualità dell'evidenza è stata bassa.

Evidenza indiretta: cambiamento della gestione clinica

Tre studi trasversali hanno valutato l'impatto dei test per MMA e vitami-na B12 sulle decisioni di gestione clinica (n = 608). Non è stato indivi-duato alcuno studio relativo ai test per l'oloTC. Due di questi studi giun-gevano alla conclusione che i pazienti ospedalizzati che ricevevano esiti dei test da cui risultano bassi livelli di vitamina B12 o MMA non erano gestiti in conformità con le raccomandazioni. Uno studio ha indicato che l'MMA era stato oggetto di un follow-up adeguato nelle cliniche di medi-cina generale danesi, per quanto sia stato spesso utilizzato come test di screening nei pazienti asintomatici. Gli studi presentavano un elevato rischio di bias.

Evidenza indiretta: efficacia del cambiamento della gestione clinica

Otto RS hanno esaminato la supplementazione di vitamina B12 nei pa-zienti con una carenza di questa vitamina. Gli studi hanno esaminato popolazioni con carenza di vitamina B12 correlata a disturbi gastrointe-stinali, anemia perniciosa, decorso pre- e/o postoperatorio di interventi di chirurgia bariatrica, disturbi neurodegenerativi e/o deterioramento cognitivo, oppure di eziologia sconosciuta. Questi studi hanno esamina-to le differenze tra il dosaggio e la via di supplementazione della vitami-na B12. Nei partecipanti con livelli deficitari di vitamina B12, dosi elevate (ossia 1000 µg) di tale vitamina somministrate per via orale hanno evi-denziato un'efficacia pari alla somministrazione per via intramuscolare al fine di correggere la carenza di vitamina B12 e conseguire risposte clini-che. Le revisioni non hanno valutato la sicurezza della supplementazio-ne. Il rischio di bias nelle revisioni incluse si è collocato in un intervallo da basso a elevato.

Cinque RS e 30 studi primari di ricerca hanno indagato il decorso natu-rale della carenza di vitamina B12. Tali studi hanno evidenziato che, se non trattati, i livelli insufficienti di vitamina B12 possono tradursi in pato-logie di grado da medio a grave (ad es. anomalie neurologiche, deterio-ramento cognitivo, anomalie ematologiche, complicazioni durante la gravidanza).

Questioni di natura sociale, legale, etica e organizzativa

Indagini sistematiche e non sistematiche hanno individuato 22 studi rile-vanti di ricerca primaria e secondaria. Le problematiche sociali e orga-nizzative correlate ai test della vitamina B12 comprendevano la medica-lizzazione delle vitamine e il conseguente aumento dello svolgimento di test non necessari. I medici hanno raccomandato diversi modi per ridur-re nella

pratica i test della vitamina B12 non necessari: ulteriori risorse di formazione e linee guida aggiornate, tempi di consultazione più estesi, rimozione degli esami della vitamina B12 dai kit di test. Una primaria fonte di preoccupazione sul piano etico è stata correlata ai falsi positivi e ai falsi negativi; tuttavia, il danno potenziale causato da questi esiti errati risulta minimo, in quanto occorrono molti anni per raggiungere una de-plezione dei livelli di vitamina B12 e la carenza vitaminica è trattabile mediante supplementazione, che i medici curanti tendono a prescrivere per i casi limite. Non sono state individuate problematiche di natura le-gale.

Conclusione

Non sussiste attualmente alcuna evidenza che misura direttamente l'impatto dei test per la vitamina B12 sui risultati riportati per i pazienti. In maniera analoga, nella prassi svizzera non sembrano esistere standard clinici ampiamente accettati per le indicazioni, per il numero e la sequenza dei test, nonché per le soglie diagnostiche dei test per la vitamina B12. La valutazione dell'accuratezza diagnostica di questi test è complicata dall'assenza di uno standard di riferimento affermato, dalla presenza di fattori di confusione (ad es. insufficienza renale, età) e dalla variabilità delle soglie di riferimento per la diagnosi. L'attuale evidenza non fornisce indicazioni circa il test più appropriato ovvero la combinazione di test più appropriata per condurre indagini su un paziente con una sospetta carenza o un elevato rischio di carenza di vitamina B12, né circa l'utilità di tali test al fine di produrre un impatto sui risultati rilevanti per il paziente.

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Abbreviations and acronyms

AMSTAR	A MeaSurement Tool to Assess systematic Reviews
CHF	Swiss francs
CI	Confidence interval
CLIA	Chemiluminescent immunoassay
CoA	Coenzyme A
FOPH	Federal Office of Public Health (Switzerland)
GI	Gastrointestinal
GP	General practitioner
GRADE	Grading of Recommendations, Assessment, Development and Evaluations
Hcy	Homocysteine
HoloTC	Holotranscobalamin II
HTA	Health technology assessment
IF	Intrinsic factor
IHE	Institute of Health Economics
IM	Intramuscular
MCV	Mean corpuscular volume
MMA	Methylmalonic acid
MTP	Microbiological tests with microtitre plates
NTD	Neural tube defect
PICO	Population, intervention, comparator, outcome
P-B12/MMA/Hcy/holoTC	Plasma vitamin B12/MMA/Hcy/holoTC
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
QUADAS	Quality Assessment of Diagnostic Accuracy Studies
RCT	Randomised controlled trial
SIGN	Scottish Intercollegiate Guideline Network
SR	Systematic review
S-B12/MMA/Hcy/holoTC	Serum vitamin B12/MMA/Hcy/holoTC
T-B12/MMA/Hcy/holoTC	Total vitamin B12/MMA/Hcy/holoTC
UK	United Kingdom
USA	United States of America
WHO	World Health Organization

Objective of the HTA short report

The objective of a health technology assessment (HTA) short report is to generate a focused assessment of various aspects of a health technology. The HTA short report format does not include a scoping phase. The analytic methods applied to assess the value of using a health technology are described. The analytical process is systematic and transparent, and it involves multiple stakeholders. The domains covered may include clinical effectiveness and safety, and legal, social, ethical and organisational issues. The purpose is to inform health policy and decision-making to promote an efficient, sustainable, equitable and high-quality health system.

1 Policy question and context

In the past five years, there has been a marked increase in the use of vitamin B12 testing in Switzerland, specifically in relation to tests for vitamin B12, transcobalamin II and III, holotranscobalamin (holoTC), and methylmalonic acid (MMA). The accompanying rise in expenditure for these tests, covered under mandatory health insurance, rose from CHF 39.6 Mio in 2015 to CHF 65.2 Mio in 2019. It has been questioned whether vitamin B12 testing in patients at risk of B12 deficiency, and in patients with a clinical suspicion of B12 deficiency, is essential prior to commencing vitamin B12 supplementation.

2 Medical background

2.1 Medical context of the technology/disease

2.1.1 Function and sources of vitamin B12

Vitamin B12, also known as cobalamin, is a water-soluble compound with roles in the production of red blood cells, synthesis of thymidine for generation of deoxyribonucleic acid, and maintenance of the myelin coating of neurons.[1] Vitamin B12 contains the metal ion cobalt, and hence 'cobalamin' is used to refer to compounds that have vitamin B12 activity.

Humans depend on the dietary intake of vitamin B12, with sufficient amounts found naturally in animal-derived food products such as meat, poultry, fish, shellfish, eggs, milk, and milk products.[2 3] Vitamin B12 is not commonly found in plant-based foods,[4 5] although various types of edible algae (i.e. nori) and nutritional yeast have been found to contain substantial amounts of vitamin B12.[6] Trace levels of vitamin B12 have also been detected in fermented beans and vegetables, along with edible mushrooms, beans, and vegetables grown in soil enriched using organic fertilisers or hydroponics.[6] Fortified foods such as breakfast cereals are a readily available source of vitamin B12, often providing an important proportion of daily intake.[3 7] Examples of sources of dietary vitamin B12 are presented in ***Table 1***.

To prevent and treat a vitamin B12 deficiency, vitamin B12 can also be supplemented via intramuscular, oral, and nasal routes.[8] A number of formulation of supplemental vitamin B12 exist, including methylcobalamin, hydroxocobalamin, and cyanocobalamin. The use of each formulation differs depending on the condition or symptoms being treated.

Table 1 Dietary sources of vitamin B12

Dietary source	Serving size; cooking method	Estimated vitamin B12 content (µg)
Clams (mixed species)	85g, cooked	84.1
Liver (beef)	85g, cooked	70.7
Mackerel (king)	85g, cooked	15.3
Breakfast cereals (fortified)* e.g. Kellogg's All-bran complete wheat flakes	1 serve	6.1
Trout (rainbow, farmed)	85g, cooked	3.5
Salmon (sockeye)	85g, cooked	4.8
Beef (top sirloin)	85g, broiled	1.4
Milk (low fat)	236ml (1 cup)	1.2
Cheese (Swiss)	28g	0.9
Eggs	1 whole, hard boiled	0.6
Chicken (breast meat)	85g, roasted	0.3

Abbreviations
g = grams, **µg** = micrograms.
Notes
*The estimated vitamin B12 content in fortified breakfast cereals varies considerably.
Source
Adapted from National Institute of Health 2020[9] and US Department of Agriculture 2017[10]

2.1.2 Mechanism of vitamin B12 absorption

Salivary glands and stomach parietal cells excrete the protein haptocorrin, which forms a complex with dietary vitamin B12. This binding protects the vitamin from degradation by stomach acid and proteases, allowing it to move through the gastrointestinal tract to the terminal ileum. Here, pancreatic enzymes release the vitamin from the protein. The vitamin is subsequently available to bind to the intrinsic factor protein (IF), which transports vitamin B12 into the bloodstream for distribution around the body. After its release into the bloodstream, the vitamin binds to the protein transcobalamin II, becoming holoTC, which is considered the 'active' B12 form.[11] HoloTC can then be transported into the cells for metabolism. Alternatively, the vitamin binds to haptocorrin and is not taken up by cells. This is the case for approximately 80% of absorbed vitamin B12.[12] Interruption of any of these steps places a person at risk of developing vitamin B12 deficiency. However, stores of excess vitamin B12 in the liver may take several years to deplete before ultimately resulting in a deficiency.[3]

An IF-independent pathway results in the passive absorption of a small amount of dietary vitamin B12 (approximately 1%).[13] As demonstrated by untreated individuals with abnormal IF production or function, malabsorption patients cannot exclusively rely on passive diffusion long term.

2.1.3 Causes of vitamin B12 deficiency

A deficiency is defined as a lack of vitamin B12 within cellular/metabolic processes that may cause symptoms and morbidities if untreated. The term 'deficiency' describes the condition independent of its aetiology (i.e. malnutrition or malabsorption). Within this report, cases with low vitamin B12 intake (e.g. vegan diet) are referred to as malnutrition, and cases with low/poor absorption (e.g. post-bariatric surgery) are referred to as malabsorption.

There are a variety of causes that lead to vitamin B12 deficiency within specific high-risk groups. The likelihood of developing vitamin B12 deficiency increases with age, with adults older than 60 years at the greatest risk.[14] In this group, the primary cause of vitamin B12 deficiency is pernicious anaemia and malabsorption of food-bound vitamin B12.[15] Other groups at risk of dietary deficiency include those who do not consume animal-based food products (particularly pregnant and lactating women).[15 16] In less common cases, deficiency may be due to hereditary vitamin B12 metabolic diseases leading to the inability to metabolise and absorb vitamin B12.

Malabsorption of food-bound vitamin B12

Malabsorption of food-bound vitamin B12 is characterised by the inability to absorb food- and/or protein-bound vitamin B12.[17] Malabsorption of food-bound vitamin B12 is most often caused by atrophic gastritis,[17] a chronic inflammatory condition characterised by atrophy of the oxyntic glands, which results in reduced secretion of gastric acid and IF.[3] Atrophic gastritis commonly leads to diminished gastric function and can result in bacterial overgrowth in the stomach and small intestine, leading to vitamin B12 malabsorption.[14] Atrophic gastritis affects 10% to 30% of adults over 60 years of age.[14]

Another cause of malabsorption of food-bound vitamin B12 is pernicious anaemia, an autoimmune disease occurring in end stage atrophic gastritis.[18 19] Pernicious anaemia is the result of autoimmune antibodies that destroy parietal cells in the stomach or bind to IF, thereby preventing its interaction with vitamin B12 or its receptor on the ileum.[20] The destruction of these cells and blocking of IF causes decreased secretion of acid and enzymes crucial in releasing food-bound vitamin B12, ultimately leading to malabsorption.[18] Pernicious anaemia affects approximately 1% to 2% of adults over 60 years of age.[21]

Both atrophic gastritis and pernicious anaemia have also been associated with *Helicobacter pylori* infection,[9 18 22] a bacterium causing chronic inflammation of the stomach, which can progress to peptic ulcer disease and gastric cancer, and can also lead to increased chances of having gastric surgery.[8 9 22]

Other less common causes of vitamin B12 deficiency resulting from malabsorption include alcoholism, and the use of drugs such as antacids (i.e. proton pump inhibitors and H2 receptor antagonists),

biguanides (i.e. metformin), aminoglycoside, antibiotics, and colchicine.[4 6 23] Gastrointestinal (GI) surgery, including gastric resection and bariatric surgery, may cause the loss of cells that secrete IF and hydrochloric acid,[24] resulting in a reduced amount of vitamin B12 able to be absorbed from foods.[24] Other GI disorders such as Crohn's disease, Whipple's disease and coeliac disease may also result in vitamin B12 deficiency due to the inability to absorb food-bound vitamin B12.[25]

Dietary deficiency

Vitamin B12 deficiency may arise in those who consume a strict vegetarian or vegan diet due to the nonconsumption of animal-based food products, which are the main source of vitamin B12 in the human diet.[17 22] Pregnant or lactating women who follow a strict vegetarian or vegan diet are also at an increased risk of vitamin B12 deficiency.[9 26] During pregnancy, vitamin B12 crosses the placental barrier. It is also present in breast milk. Therefore, women who do not consume animal-based food products may have very low reserves of vitamin B12, placing their foetus and breastfeeding children at risk of deficiency.[9 26]

Hereditary vitamin B12 metabolism diseases

In rare cases, hereditary diseases affecting the metabolism of vitamin B12 have been found in infants. For example, Imerslund-Gräsbeck syndrome is a hereditary vitamin B12 malabsorption syndrome that causes neurological disorders and megaloblastic anaemia in affected patients.[27] Another hereditary condition with similar symptoms is found in patients with congenital pernicious anaemia (i.e. IF deficiency), leading to the inability to absorb vitamin B12 due to the lack of IF.[28]

2.2 Incidence or prevalence of the disease

There is limited published literature to inform the prevalence rates of vitamin B12 deficiency in Switzerland (***Table 23***). Therefore, the prevalence rates from other World Health Organisation (WHO)-Mortality Stratum A are presented, noting the applicability to Switzerland is uncertain. In addition, the following prevalence estimates likely underestimate the true rates as many patients may be unaware they are deficient (and hence are not tested), owing to the asymptomatic nature of the disorder.

The prevalence of vitamin B12 deficiency reported in the literature varies considerably, with 2.7% of adults in the United States of America (USA), 8.3% of adults in the United Kingdom (UK) and 14.7% of adults in Germany diagnosed with low serum or plasma B12 concentrations (less than 130 to 148 picomoles per litre (pmol/L)).[29] In Switzerland, the incidence of vitamin B12 deficiency has been reported as 8.0% in people aged 60 to 69 years, to 13.4% for those aged 70 to 79, and 19.0% for those aged 80

and older.[30] The incidence of pernicious anaemia also increases with age, and is a main cause of vitamin B12 deficiency in 15% to 20% of cases.[31]

The prevalence of vitamin B12 deficiency varies among specific risk populations (***Table 2***). For example, the highest prevalence of vitamin B12 deficiency was observed in vegetarians with the lowest prevalence observed in pregnant women.[32]

Table 2 The prevalence of vitamin B12 deficiency stratified by clinical condition

Clinical condition	Prevalence rate of vitamin B12 deficiency
Achlorhydria (absence of hydrochloric acid in the stomach)	20% of those age >65 years
Vegetarianism	53% of lacto-ovo vegetarians
Crohn's disease	42% of post-surgery patients
Metformin use	10% to 30% of metformin users
Pregnancy	10% of women after 28 days pregnancy

Source
Moore 2012[32]

2.3 Natural course and/or burden of the disease

People with a clinical suspicion of vitamin B12 deficiency may have symptoms ranging from mild (e.g. fatigue) to severe (e.g. dementia and polyneuropathies).[16]

Symptoms vary among patients and are often ambiguous. This is particularly true in the elderly, and subclinical cases.[16] Individuals with B12 deficiency may also be asymptomatic and not present with symptoms for several years.[3] Symptoms and signs associated with vitamin B12 deficiency are presented in ***Table 3***.

Table 3 Symptoms and signs of vitamin B12 deficiency

Body system/organs involved	Symptoms and signs
Gastrointestinal	Glossitis
Haematological	Anaemia (macrocytic, megaloblastic)
	Leukopenia
	Pancytopenia
	Thrombocytopenia
	Thrombocytosis
Neurological and psychiatric	Areflexia
	Cognitive impairment, including dementia-like symptoms and acute psychosis
	Gait abnormalities
	Irritability
	Loss of proprioception and vibratory sense
	Olfactory impairment
Skin	Hyperpigmentation
	Jaundice
	Vitiligo

Source
Langan 2017[3]

2.4 Diagnostic and treatment pathway(s)

2.4.1 Diagnostic pathway

There are four main tests that can be performed to investigate vitamin B12 levels, but there is currently no single reference test available that can confirm a diagnosis of vitamin B12 deficiency. Each test (total serum B12, holoTC, MMA, and homocysteine) is susceptible to confounding factors. A total serum B12 test is used as a first-line test where there is cause to suspect deficiency. The result may be positive, negative or in a grey zone (i.e. an area of uncertainty between positive and negative results). A positive result or a grey zone result is commonly investigated via a second-line test that may be holoTC, MMA or homocysteine (Hcy). Performing these tests in addition to the serum B12 test increases test sensitivity and specificity of the testing cascade, ultimately reducing false positive and false negative rates.[33 34] However, diagnostic thresholds for each test vary according to different guidelines, countries, or testing laboratories (see ***Section 9*** for further information). Likewise, recommendations for these tests, and the order in which they are recommended, vary across guidelines.[16]

The diagnosis of vitamin B12 deficiency is further complicated if a patient's signs and/or symptoms do not correlate with test results. Some people with positive test results may be asymptomatic, while others who are symptomatic can have test results within the normal range.[16] Therefore, the diagnosis of vitamin B12 deficiency and subsequent treatment recommendations require a consideration of the test results and the clinical picture.[16] ***Figure 1*** depicts two possible pathways for diagnosis and treatment in patients with a clinical suspicion or higher risk of vitamin B12 deficiency.

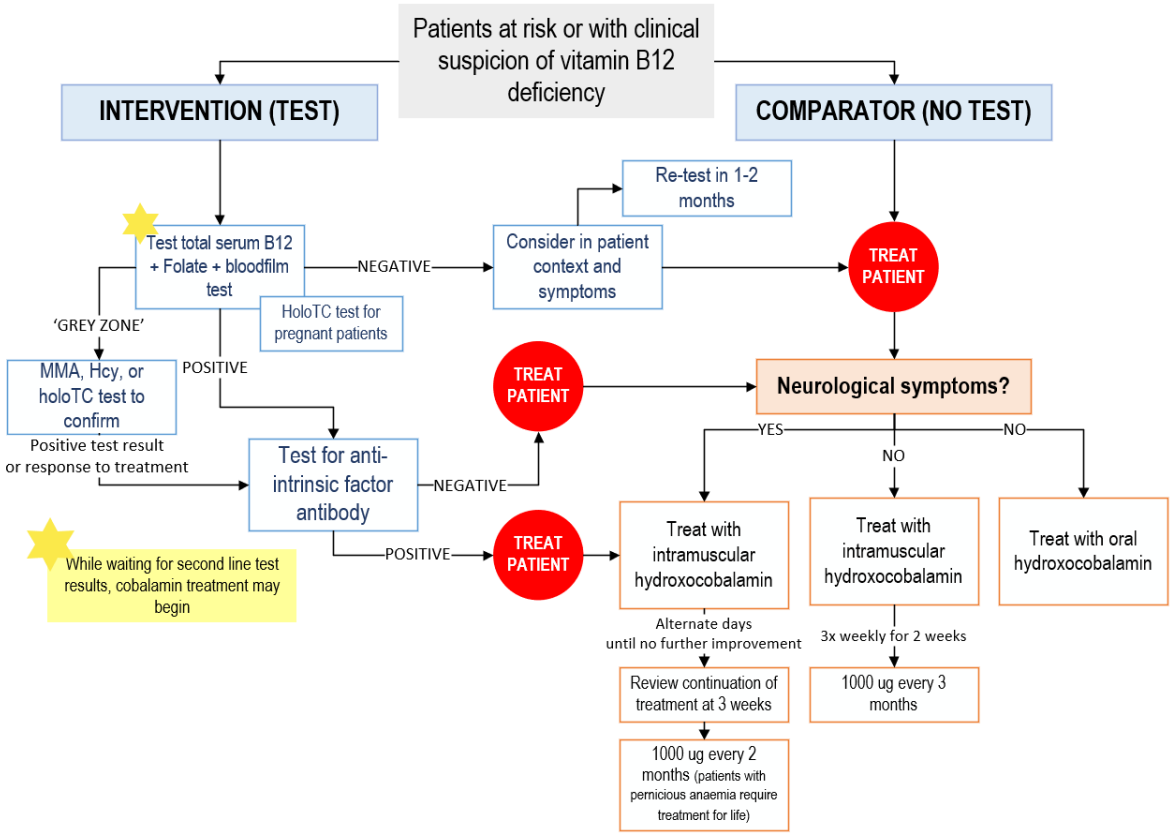

Figure 1 Pathway for diagnosing and treating patients with clinical suspicion or at risk of vitamin B12 deficiency

Abbreviations
Hcy = homocysteine, **holoTC** = holotranscobalamin II, **MMA** = methylmalonic acid, **µg** = micrograms.
Source
Guidance described in Devalia 2014[16] was used to create this figure.

Total vitamin B12

Quantifying total serum or plasma vitamin B12 is the most common and direct method used in the assessment of a suspected B12 deficiency. However, the test is susceptible to confounding factors such as genetic polymorphisms, age, and renal impairment.[34] Anti-IF antibodies in pernicious anaemia can interfere with the assay and lead to false negative or even elevated results.[35 36] Furthermore, the type of assay and method used may have an affect on accuracy.[37] Up to 80% of circulating vitamin B12 is

not available for cellular uptake.[11] It is recommended that the diagnostic results are considered in the context of the clinical situation.[11 37] The only publicly available Swiss guideline, published by MediX, suggests total serum/plasma B12 as a first-line test to investigate suspicion of vitamin B12 deficiency, or to test at-risk patients.[38]

HoloTC

Up to 80% of circulating B12 is bound to the protein haptocorrin, forming a compound known as holohaptocorrin. This binding prevents interactions between B12 and the transport protein transcobalamin II that is responsible for transporting vitamin B12 into cells for metabolism. This transport only occurs when vitamin B12 and transcobalamin II are bound, forming holoTC. Vitamin B12 testing does not differentiate between holoTC and holohaptocorrin, therefore total vitamin B12 status results often do not give a true picture of the fraction of circulating B12 that can be used in cellular metabolic processes.[11] Measuring holoTC therefore seems to be a valuable tool in the diagnosis of vitamin B12 deficiency.[11] However, the advantages of measuring holoTC instead of vitamin B12 are not consistent across the literature, with Swiss practice guidelines only recommending the use of holoTC to diagnose deficiency in pregnant women.[38] Similar to the total B12 test (***Figure 1***), holoTC has a grey zone and variable thresholds among guidelines. Recommendations against the use of holoTC as a first line test often cite the high cost of the test.[33]

MMA

MMA is a biomarker used to assess vitamin B12 tissue status. Cellular MMA is produced during the breakdown of proteins and is a crucial substrate during succinyl-CoA production and catabolism of amino acids and odd-chain fatty acids.[11] Vitamin B12 acts as a catalyst in the conversion of MMA-CoA to succinyl-CoA (see ***Figure 2***).[39 40] A reduction of activity in this pathway due to limited vitamin B12 results in an accumulation of MMA, which is regarded as a biomarker for vitamin B12 deficiency. However, this biomarker test is sensitive to factors such as renal insufficiency, age, bacterial overgrowth, hypovolaemia, and enzyme defects.[34 41] Patient context is an important consideration when deciding whether to test MMA levels. Swiss and other clinical practice guidelines suggest using MMA as a second-line test to confirm a deficiency after a low or grey zone serum B12 result.[3 38]

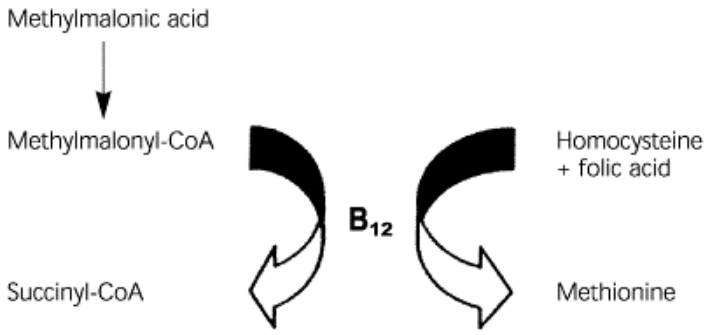

Figure 2 The role of vitamin B12 in the pathway of succinyl-CoA and methionine from MMA and homocysteine + folic acid

Abbreviations
CoA = coenzyme A, **MMA** = methylmalonic acid.
Source
Oh 200340

Other tests for B12 deficiency

Vitamin B12 and holoTC tests are the focus of this report, with some discussion of MMA testing. Two additional blood tests (i.e. Hcy and mean corpuscular value [MCV]) are mentioned in the literature and may be used by some clinicians in assessing serum B12 status. These tests are outside of the scope of this report as they are non-specific and of less relevance to the Swiss context. The Swiss guideline (MediX)[38] discusses how Hcy and MCV may be used as indicators of vitamin B12 deficiency in the laboratory, however these tests are not described in the diagnostic pathway.

- Hcy: In vitamin B12 deficiency, the metabolites MMA and Hcy accumulate as the pathway becomes limited by the unavailability of vitamin B12 (see ***Figure 2***). These products are considered to be sensitive biomarkers that are useful for indicating a vitamin B12 deficiency at the tissue level. Hcy has many confounding factors and other diseases associated with its elevation, such as folate deficiency. Consequently, it is non-specific and inaccurate when used alone. When used in combination with other tests, Hcy is comparatively more useful for detecting a vitamin B12 deficiency than when used alone.[42]
- MCV: MCV is a measure of the volume of red blood cells. A systematic review (SR) reported that up to 84% of B12-deficient patients will be overlooked if MCV is used to select patients for further evaluation (based on results obtained from an anaemic and non-anaemic population).[43]
- 4cB12: A model has been recently developed by Fedosov (2010), which incorporates all four vitamin B12 tests (serum B12, MMA, holoTC, and Hcy).[44] The values obtained from the tests, along with a patient's age, are input into a formula (depicted below). The equation can

categorise patients into groups of low or subclinical vitamin B12, and possible or probable vitamin B12 deficiency.

$$4\text{cB12} = \log 10\ \frac{\text{HoloTC x B12}}{\text{MMA x Hcy}} - \frac{3.79}{1 + (\frac{\text{age}}{230})\text{^}2.6}$$

2.4.2 Treatment pathways

Patient context is important when considering treatment options. Factors such as aetiology and symptoms influence decisions concerning the dosage, frequency, and route of drug administration.

Treatment guidelines vary among countries. The British Journal of Haematology guidelines developed by Devalia (2014) recommend that a patient with pernicious anaemia, malabsorption or experiencing neurological symptoms should receive 1,000 micrograms (µg) of intramuscular (IM) hydroxocobalamin on alternate days until no further improvement is observed, with review after 3 weeks of treatment.[16] Subsequently, the same dose is to be administered once every 2 months to maintain serum vitamin B12 levels. For other patients, administration of 1,000 µg of IM hydroxocobalamin is recommended 3 times per week for 2 weeks, followed by the same dose every 3 months as maintenance. An alternate drug, cyanocobalamin, can be delivered orally or parenterally. Hydroxocobalamin and cyanocobalamin availability and preference vary among countries.[38]

In Switzerland, patients with either pernicious anaemia, who have undergone gastric surgery, or are following a vegan diet typically receive either 1,000 µg of cyanocobalamin or 1,000 µg of hydroxocobalamin. Subcutaneous or IM administration is recommended on days 1, 3, and 5 (or days 1–5), then weekly for 1 month, followed by every 1 to 3 months.[38] Alternatively, oral therapy with high dose vitamin B12 (cyanocobalamin 1,000 µg) is also recommended.[38]

3 Technology

3.1 Technology description

Individuals at high risk of vitamin B12 deficiency, or who present with signs and symptoms indicative of vitamin B12 deficiency or pernicious anaemia, are currently advised to have their vitamin B12 levels evaluated.[3] [16] Currently, there are no restrictions around vitamin B12 testing in clinical practice in Switzerland (i.e. frequency of testing), therefore, testing is at the sole discretion of the physician. Testing of vitamin B12, holoTC, and MMA has increased significantly in Switzerland over the past decade, particularly total serum vitamin B12 (***Table 4***). Consequently, the use of vitamin B12 testing is under investigation.

Table 4 Number of claims and costs of various B12 tests in Switzerland, 2012 to 2019

	Year							
	2012	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**
Number of tests								
MMA	0	0	1	1	3	8	3,999	11,064
HoloTC	0	1	0	0	1	2	179,318	541,761
Vitamin B12	548	1,745	76,771	793,574	944,154	1.1 Mio	1.3 Mio	1.2 Mio
Transcobalamin II	135	570	25,227	316,289	385,438	475,079	342,771	448
Transcobalamin III	1	5	197	1,617	1,260	537	390	0
Total	684	2,321	102,196	1.1 Mio	1.3 Mio	1.6 Mio	1.8 Mio	1.8 Mio
Sum reimbursed (CHF) by mandatory insurance								
MMA	0	0	47	56	187	376	440,603	1.2 Mio
HoloTC	0	61	0	0	61	122	11.0 Mio	33.2 Mio
Vitamin B12	13,785	43,752	1.9 Mio	20.0 Mio	23.7 Mio	28.5 Mio	31.6 Mio	30.7 Mio
Transcobalamin II	8,235	34,770	1.5 Mio	19.4 Mio	23.6 Mio	29.0 Mio	12.0 Mio	27,328
Transcobalamin III	135	675	26,549	216,918	169,565	72,501	52,347	0
Total	22,155	79,258	3.5 Mio	39.6 Mio	47.5 Mio	57.7 Mio	64.0 Mio	65.2 Mio

Abbreviations
CHF = Swiss francs, **holoTC** = holotranscobalamin II, **MMA** = methylmalonic acid.
Source
Bundesamt für Gesundheit (BAG) 2020[45]

3.1.1 Vitamin B12 testing thresholds for diagnosing deficiency

For each test, there is no clear consensus around the most appropriate reference values and thresholds for diagnosing vitamin B12 deficiency. This lack of standardisation is likely to impact the reported diagnostic accuracy of each test in different populations and settings. Additionally, this will also influence the interpretation of test results and is therefore an important consideration when evaluating this technology. See ***Section 9***, ***Table 23*** for further details of current thresholds used to diagnose vitamin B12 deficiency.

3.2 Alternative technologies

The alternative to testing is either watch-and-wait or initiation of supplementation. A watch-and-wait approach involves monitoring a patient's symptoms. A change in management will occur when there is a change in symptoms. Alternatively, physicians may initiate supplementation if the patient has risk factors or symptoms associated with B12 deficiency. In Switzerland, vitamin B12 supplementation is typically 1,000 µg delivered intramuscularly, subcutaneously, or orally. The frequency and route of B12 supplementation is dependent on the type and severity of symptoms.[38]

3.3 Regulatory status / provider

In Switzerland, total serum B12, holoTC, and MMA testing is reimbursed under mandatory health insurance through the Analysenliste (German), Liste des Analyses (French) or Elenco delle Analisi (Italian) (***Table 5***). Testing is not restricted for total serum B12 or holoTC, however, MMA is used as a second-tier test which acts to confirm a prior vitamin B12 test.

Table 5 Tests available for the evaluation of vitamin B12 deficiency in Switzerland (Analysenliste)

Designation	Limitations	Position number	Tax point
Vitamin B12, Cyanocobalamin (i.e. serum B12)	None	1749.00	25
HoloTC	None	1727.10	61
MMA	Second tier test for vitamin B12 deficiency	1568.00	110

Abbreviations
HoloTC = holotranscobalamin II, **MMA** = methylmalonic acid.
Notes
Analysenliste Volume: 1 July 2020
Source
Bundesamt für Gesundheit BAG 2020.[45]

The regulation of the use of vitamin B12 tests vary among western countries. For example, in 2012 Ontario, Canada, delisted the vitamin B12 test.[46] In Australia (2015) limits have been placed on the reimbursement of B12 testing, whereby serum vitamin B12 testing is limited to one test per year, and the biomarkers holoTC or MMA may be tested when serum B12 is low or equivocal.[47] In North Carolina (2019) [48] MMA and vitamin B12 testing is covered, excluding under the following circumstances: Vitamin B12 and MMA testing is not covered when screening healthy or asymptomatic patients, Hcy is not covered for vitamin B12 status confirmation, and HoloTC is not covered for screening testing or confirmation of B12.

4 HTA key questions

The aim of this HTA short report was to investigate the following research question:

1. In patients with a clinical suspicion of vitamin B12 deficiency, or at high risk of vitamin B12 deficiency, is vitamin B12 (total serum B12, holoTC, and/or MMA) prior to vitamin B12 supplementation safe and effective compared to no serum B12 testing?

In the absence of direct evidence on the safety and effectiveness of vitamin B12 testing, a linked evidence approach was undertaken to investigate the following research questions:

2. In patients with a clinical suspicion of vitamin B12 deficiency, or at high risk of vitamin B12 deficiency, what is the diagnostic accuracy of vitamin B12 testing?
3. In patients with a clinical suspicion of vitamin B12 deficiency, or at high risk of vitamin B12 deficiency, does vitamin B12 testing prior to vitamin B12 supplementation change patient management (i.e. the commencement, route and frequency of supplementation), compared to no testing?
4. Do changes in patient management as a result of vitamin B12 testing impact patient outcomes?
    a. Do alterations in management impact patients with vitamin B12 deficiency (i.e. true positives)?
    b. Do alterations in management impact patients suspected of vitamin B12 deficiency who are incorrectly diagnosed (i.e. false positive and false negatives)?

5 Methodology

5.1 Literature search

5.1.1 Databases and other sources

The review methods were defined *a priori* in a protocol that underwent independent, anonymous peer review. Systematic literature searches were conducted to identify (a) existing SRs (search date up to 18 October 2020), and (b) primary studies addressing gaps in existing SRs addressing the research questions (search date up to 4 November 2020). The searches were performed in five biomedical databases (Medline, EMBASE, Cochrane Library, York CRD, INAHTA HTA database). For the SR searches, a methodological search filter published by the Canadian Agency for Drugs and Technologies in Health was used to limit the search results by publication type.[49] In addition, grey literature searches were conducted on HTA agency and specialty websites. The search strategies are presented in ***Appendix A*** (***Table 26*** to ***Table 37***).

5.1.2 Inclusion and exclusion criteria

The population, intervention, comparator, outcome (PICO) criteria for studies evaluating the direct effectiveness and safety of B12 testing is outlined in ***Table 6***. No additional limits were used.

Table 6 Inclusion criteria for direct evidence of vitamin B12 testing effectiveness (Research Question 1)

Research question	In patients with a clinical suspicion of, or at high risk for, vitamin B12 deficiency, is vitamin B12 testing prior to vitamin B12 supplementation safe and effective compared to no testing?
Population	Patients with a clinical suspicion of vitamin B12 deficiency e.g. macrocytosis, dementia, paraesthesia, polyneuropathy, glossitis, malnutrition. Patients in high-risk populations e.g. post-bariatric surgery or gastric resection, vegans, vegetarians, age 65+.
Intervention	Diagnostic serum testing (total serum B12, holoTC, MMA) for vitamin B12 deficiency.
Comparator	No testing.
Outcomes	Safety: Adverse events from testing (blood draw) or treatment (oral, nasal or intramuscular). Effectiveness: Improvement of symptoms, signs, or quality of life within a defined period (e.g. anaemia, neurological symptoms) and acceptable serum vitamin B12 levels established, or prevention of deficiency development within a defined period.
Study designs	Safety: SRs, RCTs, prospective non-RCTs. Effectiveness: SRs and RCTs.
Language	Study selection will be limited to studies published in English and those in French, German, and Italian if accompanied by English language abstracts.
Country	WHO-Mortality-Stratum A countries.[50]
Year	No date limits were applied.

Abbreviations
HoloTC = holotranscobalamin II, **MMA** = methylmalonic acid, **RCT** = randomised controlled trial, **SR** = systematic review, **WHO** = World Health Organization.

As direct evidence was not available, a linked evidence approach was undertaken to evaluate the impact of vitamin B12 testing on patient outcomes. The first step in the linked evidence approach was to investigate the performance of the test (***Research Question 2***, ***Table 7***). Subsequently, the impact of the test outcomes on patient management was evaluated (***Research Question 3***, ***Table 8***). The final step in the linked evidence approach was to investigate the impact of any potential changes in management on patient outcomes (***Research Question 4***, ***Table 9***). No additional limits were used.

Table 7 Inclusion criteria for the diagnostic accuracy of vitamin B12 testing (Research Question 2)

Research question	In patients with a clinical suspicion of, or at high risk of, vitamin B12 deficiency, what is the diagnostic accuracy of vitamin B12 testing?
Population	Patients with a clinical suspicion of vitamin B12 deficiency e.g. macrocytosis, dementia, paraesthesia, polyneuropathy, glossitis, malnutrition. Patients in high-risk populations e.g. post- bariatric surgery or gastric resection, vegans, vegetarians, age 65+.
Presentation	Symptoms of B12 deficiency. Asymptomatic but with risk factors for B12 deficiency.
Index tests	Any diagnostic screening/test of vitamin B12 (total serum B12, holoTC, MMA).
Comparator tests	Any diagnostic screening/test of vitamin B12 (total serum B12, holoTC, MMA).
Purpose	To quantify vitamin B12 levels to diagnose symptomatic or asymptomatic B12 deficiency. To evaluate the index test as a triage test where those who test positive undergo a second test to obtain a more accurate diagnosis.
Outcome	Test accuracy: Sensitivity, specificity, positive/negative predictive values, false positives, false negatives, invalid/uninterpretable results, positive predictive value, negative predictive, value confirmed by a valid reference test.
Reference standard	No accepted reference test for vitamin B12 deficiency. To validate diagnostic accuracy results, any other test of vitamin B12 may be used e.g. total serum B12, holoTC, MMA or clinical monitoring of symptoms.
Study designs	SRs of diagnostic accuracy studies. If no SRs were available primary diagnostic accuracy studies will be considered.
Language	Study selection will be limited to studies published in English and those in French, German, and Italian if accompanied by English language abstracts.
Country	WHO-Mortality-Stratum A countries.[50]
Year	Primary studies published post-search period in SR by Willis (November 2009).

Abbreviations
HoloTC = holotranscobalamin II, **MMA** = methylmalonic acid, **WHO** = World Health Organization.

Table 8 Inclusion criteria for the impact of testing on clinical management (Research Question 3)

Research question	In patients with a clinical suspicion of, or at high risk for, vitamin B12 deficiency, does vitamin B12 testing prior to vitamin B12 supplementation affect supplementation commencement, route or frequency compared to no testing?
Population	Patients with a clinical suspicion of vitamin B12 deficiency e.g. macrocytosis, dementia, paraesthesia, polyneuropathy, glossitis, malnutrition Patients in high-risk populations e.g. post- bariatric surgery or gastric resection, vegans, vegetarians, age 65+
Intervention	Diagnostic testing (total serum B12, holoTC, MMA) for vitamin B12 deficiency.
Comparator	No testing.
Outcomes	% change in number of people treated with supplementation. % change in number of people who opt for oral vs parenteral/intramuscular injection therapy. % change in number of patients with a condition other than vitamin B12 deficiency that undergo unnecessary treatment.
Study designs	SRs, RCTs, non-randomised studies, single-arm trials (before and after).
Language	Study selection will be limited to studies published in English and those in French, German, and Italian if accompanied by English language abstracts.
Country	WHO-Mortality-Stratum A countries.[50]
Year	No date limits were applied.

Abbreviations
HoloTC = holotranscobalamin II, **MMA** = methylmalonic acid, **WHO** = World Health Organization.

Table 9 Inclusion criteria for the impact of changes in management on patient outcomes (Research Question 4)

Research questions	Do alterations in management have an impact on patients with vitamin B12 deficiency (i.e. true positives and true negatives)? Do alterations in management have an impact on patients suspected of vitamin B12 deficiency who are incorrectly diagnosed (i.e. false positives and false negatives)?
Population	Patients with a clinical suspicion of vitamin B12 deficiency e.g. macrocytosis, dementia, paraesthesia, polyneuropathy, glossitis, malnutrition Patients in high-risk populations e.g. post- bariatric surgery or gastric resection, vegans, vegetarians, age 65+.
Intervention	Vitamin B12 supplementation.
Comparator	No treatment.
Outcomes	Safety: Adverse effects. Effectiveness: Quality of life (improved or maintained), time to administration of appropriate method of supplementation, % change in supplement compliance. Disease natural course: Natural progression of condition when untreated (morbidity).
Study designs	SRs and meta-analyses of RCTs or non-randomised studies
Language	Study selection will be limited to studies published in English and those in French, German, and Italian if accompanied by English language abstracts.
Country	WHO-Mortality-Stratum A countries.[50]
Year	No date limits were applied.

Abbreviations
HoloTC = holotranscobalamin II, **MMA** = methylmalonic acid, **WHO** = World Health Organization.

5.2 Study selection

Studies were selected in accordance with the four separate PICO boxes presented in ***Table 6*** *to* ***Table 9*** relating to (a) direct evidence on the impact of testing on clinical outcomes, (b) diagnostic accuracy, (c) change in management, and (d) effectiveness of the change in management. For the selection of SRs, two independent reviewers assessed all citations by title and abstract using Rayyan (Qatar Computing Research Institute),[51] with disagreements settled by consensus. For the selection of primary studies, the first 300 studies were assessed by two reviewers to determine inter-rater reliability. Disagreement of <5% was defined *a priori* as an acceptable cut-off to justify splitting the study selection of the remaining articles by title and abstract. All articles deemed potentially relevant were then reviewed by full text by the same two reviewers independently (VF and LA), with disagreements settled by consensus. Where consensus could not be reached, conflicts were resolved by a third reviewer (TV). Case series including less than 10 participants were excluded.

5.3 Data extraction

One reviewer independently extracted data into a standardised template, which was checked against the original study record by a second reviewer. Disagreements were settled by discussion, or by a third independent reviewer where consensus could not be reached. Data of interest included:

- Study information: country, year, number of institutions, study design, inclusion/exclusion criteria, withdrawals, length of follow-up, any noteworthy features or limitations in the study.
- Demographic information: number of participants, age, sex, comorbidities, indication.
- Intervention and comparator: type and method of intervention/comparator (including index test, type, dose and administration route of the supplement), concomitant interventions.
- Outcomes of interest: baseline, final or change from baseline scores in any of the pre-defined outcomes outlined in ***Table 6*** to ***Table 9***. For the purposes of this report, sensitivity and specificity were the primary diagnostic outcomes of interest.

A contingency table defining relevant diagnostic accuracy outcomes is presented in ***Table 10***.

Table 10 Contingency table for assessment of diagnostic accuracy of tests

		Reference standard		Measures
		Positive	**Negative**	
Test result	**Positive**	True positive (TP)	False positive (FP)	Positive predictive value TP/TP+FP
	Negative	False negative (FN)	True negative (TN)	Negative predictive value TN/FN+TN
Measures		Sensitivity TP/TP+FN	Specificity TN/FP+TN	Diagnostic accuracy TP+TN/TP+FP+FN+TN

Abbreviations
FN = false negative, **FP** = false positive, **TN** = true negative, **TP** = true positive.

5.4 Risk of bias assessment

Two independent researchers conducted the risk of bias assessments, with differences settled via consensus, or by an independent reviewer where consensus could not be reached. Risk of bias was assessed by different tools depending on the study design. Included SRs were appraised using the A MeaSurement Tool to Assess systematic Reviews (AMSTAR) checklist;[52] Scottish Intercollegiate Guidelines Network (SIGN) case control quality checklist,[53] and cohort study quality checklist;[54] diagnostic accuracy studies were appraised using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) tool;[55] single-arm trials by the Institute of Health Economics (IHE) quality appraisal checklist for case series.[56] Several items in the IHE checklist were modified to be more applicable to studies of the natural course of disease. These changes are outlined in ***Section 6.4.4***.

5.5 Data synthesis

De novo meta-analysis was explicitly beyond the scope of this HTA short report. The results of existing reviews and primary studies included in the review have been summarised narratively. The overall strength of evidence for outcomes relating to comparative interventions were evaluated using the GRADE approach.[57]

6 Clinical effectiveness and safety

6.1 Summary statement clinical effectiveness and safety

Direct evidence

No direct evidence investigating the effectiveness of B12 testing was identified.

Linked evidence

Diagnostic accuracy

The sensitivity of the vitamin B12 test against MMA as a reference standard ranged from 52% to 72%, reported by one SR and meta-analysis (k = 36), and three additional primary studies (n = 2,998). These studies included patients with a suspected vitamin B12 deficiency. Two studies reported that 19% and 24% of patient had low B12 of an elevated biomarker (n = 1,719), and one study reported 69% of participants fell into the grey zone of holoTC (n = 1,279). One study exclusively included patients with neurological symptoms. Specificity ranged from 41% to 81%. A primary study conducted in Switzerland with a large sample size of participants who had concomitant results for serum B12, MMA, holoTC, and Hcy tests (n = 11,833, of which 9% were low or deficient of B12). They used a novel reference standard (4cB12 method) and reported sensitivity of 86% and specificity of 78% in identifying patients with subclinical vitamin B12 deficiency.

Three primary studies that investigated holoTC against MMA as a reference standard reported test sensitivity ranging from 56% to 83%, and specificity from 51% to 60%. The Swiss study that employed the 4cB12 model as a reference standard reported sensitivity and specificity of 86% and 81%, respectively, in possibly deficient cases.

MMA was investigated as an index test by the Swiss study using the 4cB12 reference standard, and a second primary study using holoTC as a reference standard in patients with suspected deficiency from a natural medicine clinic (n = 77). These studies reported sensitivity ranges between 40% to 82%, and specificity ranges between 83% to 94% in subclinical cases.

Evidence included in the SR and meta-analysis were evaluated as having a high risk of bias. The six primary studies were generally low risk to unclear. Domains performing poorly were typically in patient selection and reference standard. GRADE was also used to assess the quality of the evidence for the Swiss study. The evidence was very low in respect to serum B12 and low for holoTC and MMA.

Change in management

Two studies evaluated the impact of B12 testing on patient management and concluded that hospital patients who received a B12 test indicating low levels of B12 or MMA were not managed according to recommendations based on the test result. One study reported that MMA was used appropriately in Danish general practice clinics. Reference values for MMA were 0.05-0.37 µmol/L for samples collected up until 30 June 1996 and 0.08-0.28 µmol onwards. The cut off for serum B12 was <200 pmol/L. No studies regarding holoTC testing were identified. The threshold used for serum vitamin B12 deficiency was <184 pmol/L[58] or <200 pmol/L.[59 60] For MMA, a deficiency was defined as >0.37 micromoles per litre (µmol/L) when tested prior to June 30 1996, and >0.28 µmol/L afterwards.[59 60]

The studies were appraised against the IHE case series checklist. Generally, studies were evaluated as having a low to moderate risk of bias.

Effectiveness of change in management

In patients that are correctly identified as B12 deficient, B12 supplementation was found to be effective at achieving a clinical response. Cases that are incorrectly classified as healthy (i.e. false negatives) could face mild to severe consequences (e.g., neurological abnormalities, cognitive impairment, haematological abnormalities, complications during pregnancy).

The SRs pertaining to supplementation were appraised with the AMSTAR tool. The risk of bias of the SRs ranged from low to high. The evidence base for natural history studies was appraised using a modified IHE case series checklist and SIGN quality appraisal tool. The studies generally performed with low to moderate risk of bias.

6.2 Search results

The results of the study selection process are presented in ***Figure 3***. The search results from each database are reported in ***Table 24*** to ***Table 31***, ***Appendix A***. After the removal of duplicates, 6,919 records were screened by title/abstract. A total of 287 studies were reviewed by full text, of which 13 SRs and 38 primary studies met the inclusion criteria.

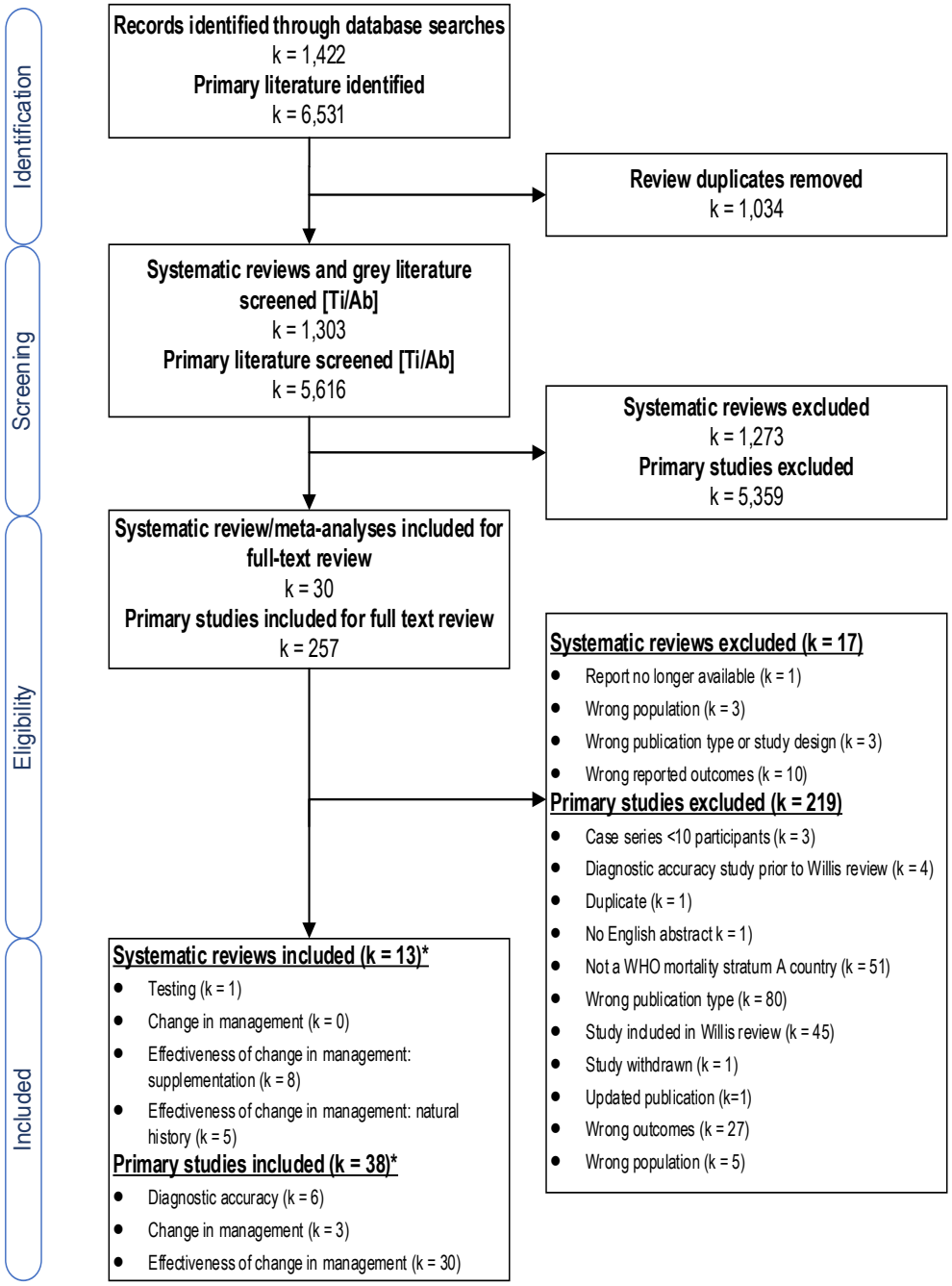

Figure 3 PRISMA flow chart for study inclusion

Abbreviations

k = number of studies, **SR** = systematic review, **Ti/Ab** = review by title and abstract, **WHO** = World Health Organization.

Notes

*One SR and one primary study were included in multiple research questions

"Willis review" refers to a systematic review by Willis (2011) that acted as the foundation for the diagnostic accuracy section of the HTA short report.[34]

6.3 Study Characteristics

In the absence of direct evidence, a linked evidence approach was followed. The study characteristics of such studies are described below.

6.3.1 Diagnostic accuracy

A single SR (including meta-analysis) was included for diagnostic accuracy. This SR, authored by Willis (2011),[34] searched for studies published between 1990 and November 2009 across 7 databases (PubMed, EMBASE, CINAHL/PsychINFO, Wed of Sciences, Current Contents and Informit Health Databases) see ***Table 38***. The review included 54 primary studies, of which 36 reported outcomes appropriate for meta-analysis. The included studies were stratified within subgroups of clinical indication (patients with neuropsychiatric symptoms, previous vitamin B12 deficiencies, no defined indications, and other) and assay method (chemiluminescence, enzyme immunoassay, radioassay, radioimmunoassay, microbiological assay).

Of the 54 studies included in the Willis SR, 13 were two-gated[24 61-72] and 41 were single-gated studies.[37 73-112] The mean or median age of the patients ranged from 31–80 years. Participants were older than 60 years in 71% (k = 48) of studies reporting on age. Age and sex were reported in 89% and 83% of studies, respectively. Men comprised 55% (k = 45) of participants among included studies. Patients with a suspected vitamin B12 deficiency due to a prior investigation comprised 33% (k = 18) of studies. Studies enrolling patients with unknown vitamin B12 status were also included (39%, k = 21). The clinical indication was not specified in 48% (k = 26) of included studies. Of the studies that presented clinical information, 36% (k = 10) of studies included patients with suspected or prior vitamin B12 deficiency, 25% (k = 7) of studies with neuropsychiatric (suspected or confirmed) conditions, 11% (k = 3) patients with gastric disorders, and 29% (k = 8) of studies with some other clinical indication. In 41% of studies, patients were enrolled in studies without knowledge of their vitamin B12 status.

The studies were based in Australia (k = 2),[64 65] Belgium (k = 2)[61 99] Canada (k = 1),[104] Denmark (k = 4),[88 89 97 98] France (k = 1),[73] Germany (k = 3),[70 86 101] Hong Kong (k = 1),[90] India (k = 1),[105] Jordan (k = 1),[83] New Zealand (k = 1),[91] Norway (k = 2),[75 87] South Africa (k = 1),[71] Sweden (k = 7),[66-69 93 94 111] the Netherlands (k = 1),[110] UK (k = 6),[72 81 82 84 95 112] and the USA (k = 19).[24 37 62 63 74 76 78-80 85 92 96 100 102 103 106-109] One study did not specify the country of origin.[77] The included studies used MMA (k = 18), Hcy (k = 18), HoloTC (k = 4), or clinical reference (k = 6) as reference tests. Citations were not provided for the studies included in the meta-analysis. The remaining included studies used MMA/creatinine ratios, or various combinations of Hcy, MMA, and holoTC. All studies included in the SR by Willis (2011)[34] used serum B12 as the index test. Various thresholds were used across the included studies, which are outlined in ***Table 45***. All primary studies used vitamin B12 as an index test,[33 113-117] all but one[116] looked at holoTC as an index test, and three studies also analysed MMA as an index test.[33 116 117]

In total, 6 additional diagnostic test accuracy studies (n = 15,608 patients) published after the search date of the Willis (2011)[34] review were included in the present review. The studies were prospective (k = 4)[113 114 116 117] or retrospective (k = 2).[33 115] The diagnostic test accuracy studies were based in Switzerland (k = 1),[33] the Netherlands (k = 1),[113] UK (k = 1),[117] or Germany (k = 3).[114-116] One study did not report any patient demographics besides age.[114] Across the remaining five studies, 50% of included patients were male.[33 113 115-117] All patients were aged between 19 to 100 years. The mean age was 66 years across 4 studies[113 115-117] and the median age was 60 years across two studies.[33 114] One study was conducted at a natural medicine clinic, which had a notably high enrolment of vegan and vegetarian participants.[116] The remaining studies did not report on the ratios of vegetarian and vegan patients to omnivorous patients. The clinical indication for the studies included samples referred for vitamin B12 determination,[33 114] neurological or cognitive signs or symptoms of deficiency,[115-117] or individuals tested for moderate, low or very low B12.[113] Diagnostic thresholds for each test were variable among the included primary studies included in this report, and in the Willis review (see ***Appendix B***, ***Table 45***). Mean or median vitamin B12, holoTC, or MMA levels of the included participants have been summarised in ***Table 11***. The majority of the included participants did not have measurements suggesting a vitamin B12 deficiency as indicated by the respective reference test. The prevalence of vitamin B12 deficiency in Switzerland is unknown. One study that investigated older people, found that the prevalence was 8%, 13%, and 19% in patients who were in their 60s, 70, and 80s respectively.[30] Index and reference tests for each included primary study are presented in ***Table 15***.

Table 11 Vitamin B12, holoTC, and MMA measurements of participants included in primary diagnostic accuracy studies

Study	Vitamin B12 (pmol/L)	HoloTC (pmol/L)	*MMA (µmol/L)*	Proportion low/deficient by reference test (%)
Campos 2020[33]†	292.0 (222-393)	64.0 (45-93)	0.175 (0.137-0.234)	8.7
Heil 2012[113]	207.5 (56.2-871)	32.5 (2.9-213.8)	0.250 (0.09-2.24)	13.1 (>0.45 MMA)
Herrmann 2013[114]	299.2	61.1	>0.300	18.6% (patients with normal creatinine) 14.1% (total cohort)
Schrempf, 2011[115]	289.0 (± 248.7 SD)	49.5 (± 26.1 SD)	0.298 (± 0.35 SD)	14.8% (>0.47 MMA)
Schwarz 2015[116]†	CLIA: 245.7 (181.9-326.1) MTP: 316.1 (244.9-398.6)	59.0 (40.3-84.5)	0.196 (0.138-0.271)	17.3%
Valente 2011[117]	254.0	47.0	0.347	8.1% (abnormal holoTC) 8.0% (abnormal B12) 41.7% (abnormal MMA >0.36)

Abbreviations
B12 = serum or plasma vitamin B12, **CLIA** = chemiluminescent immunoassay, **holoTC** = holotranscobalamin II, **MTP** = microbiological tests with microtitre plates, **MMA** = methylmalonic acid, **pmol/L** = picomoles per litre, **µmol/L** = micromoles per litre.
Notes
Values presented as mean (range) unless otherwise specified.
† = Median values

6.3.2 Change in management

Three cross-sectional studies comprising 608 participants (51% male)[58-60] investigated changes in patient management in response to vitamin B12 (k = 1) [58] or MMA test results (k = 2).[59 60] No studies investigated the impact of holoTC testing on management decisions. The studies were located in Denmark (k = 2)[59 60] and USA (k = 1).[58] The median ages were 77[59] and 81 years[60] (ranging from 19–98 years). Carmel (1986) provided limited data on age,[58] instead reporting that 45% of participants were aged >60 years, 31% aged 40–60 years, and 24% of participants aged <40 years.[58] The threshold for serum vitamin B12 deficiency was <184 pmol/L[58] or <200 pmol/L.[59 60] For MMA, a deficiency was defined as >0.37 micromoles per litre (µmol/L) when tested prior to June 30 1996, and >0.28 µmol/L afterwards.[59 60] Carmel only investigated the serum B12 test.[58]

6.3.3 Effectiveness of change in management

Supplementation

The literature search identified eight SRs that reviewed the supplementation of vitamin B12 in patients with a vitamin B12 deficiency.[8 19 32 118-122] The year of publication of the included studies ranged from 2006 to 2018. The number of studies included in each SR ranged from 2 to 43 studies of varying study designs. The number of participants included in each SR ranged between 93[119] and 1,277,[122] with a total of 3,958 participants across all studies. The populations of interest included patients with vitamin B12 deficiency related to gastrointestinal (GI) disorders,[118] patients with pernicious anaemia,[19] patients before and/or after bariatric surgery,[120 122] patients with neurodegenerative and/or cognitive impairment,[32] and patients with a vitamin B12 deficiency.[8 119 122] The age of included participants varied from 23 to 92 years, however, most patients were above the age of 65.

Five of the SRs diagnosed deficiency using only serum vitamin B12 levels,[19 32 118 120 122] and three SRs used serum vitamin B12, MMA, and Hcy.[8 119 121] Four SRs compared oral vitamin B12 therapy to IM vitamin B12 therapy,[8 19 118 119] two SRs compared various doses of oral vitamin B12,[32 120] one SR compared oral supplementation to no supplementation (or a different supplementation vitamin B12 regimen),[122] and one SR compared oral and IM vitamin B12 supplementation to placebo.[121] Supplemental dosage of vitamin B12 ranged from 1,000 µg to 2,000 µg across the included studies. ***Table 12*** provides a summary of study characteristics for the included SRs on the supplementation of vitamin B12 in patients with vitamin B12 deficiency. A detailed table of the included studies on the supplementation of vitamin B12 can be found in ***Table 40***, ***Section 12.1***, ***Appendix B***.

Table 12 Effectiveness of change in management: supplementation

Author year	Indication; included studies	Databases searched & search date	Vitamin B12 deficiency criteria	Comparisons under investigation
Andres 2018[118]	Vitamin B12 treatment in patients with vitamin B12 deficiency related to gastrointestinal disorders; 39 studies	PubMed & Google Scholar January 2010 to June 2018; Cochrane Library & the ISIWeb of Knowledge (dates NA)	s-B12: <180 pmol/L	Comparison between oral vitamin B12 and IM vitamin B12 therapy.
Butler 2006[119] (distillation of a Cochrane review)	Oral vs. IM vitamin B12 for vitamin B12 deficiency; 2 studies	Medline, Embase, Cochrane library, DARE database & Lilacs December 2004	s-B12: <180 pmol/L	Comparison between oral vitamin B12 and IM vitamin B12 therapy.
Chan 2016[19]	Oral vs. IM vitamin B12 for the treatment of pernicious anaemia; 12 studies	PubMed March 31, 2016	s-B12 (cut-off not indicated)	Comparison between oral vitamin B12 and IM vitamin B12 therapy.
Mahawar 2018[120]	Oral vitamin B12 after RYGB surgery; 19 studies	PubMed September 22, 2017	s-B12 (cut-off varied across studies)	Comparison between doses of oral vitamin B12 therapy.
Moore 2012[32]	Association between vitamin B12 & cognitive impairment or dementia; 43 studies	Medline, PubMed, PsychINFO March 2011	Deficient s-B12: <150 pmol/L Low-normal s-B12: 150-250 pmol/L	Comparison between doses of oral and parenteral vitamin B12 therapy.
Smelt 2017[122]	Supplementation regimes to treat perioperative vitamin B12 deficiency in bariatric surgery; 10 studies	PubMed, Embase, Medline & Cochrane Library December 2015	s-B12 (cut-off varied across studies)	Comparison between vitamin B12 supplementation compared to no supplementation (or different supplementation vitamin B12 regimen).
Smelt 2018[121]	Vitamin B12 supplementation on haematological parameters in older people; 4 studies	PubMed, EMBASE, Web of Science, Cochrane, CENTRAL, ClinicalTrials.gov April 2016	Assessed hgb, B12, MMA, Hcy, s-folate, MCV, Hct & RBC count (cut-off varied across studies)	Comparison between vitamin B12 therapy (all forms of administration and dosage) and placebo.
Wang 2018[8] Cochrane review	Oral vs. IM vitamin B12 for vitamin B12 deficiency; 3 RCTs	CENTRAL, MEDLINE, Embase, LILACS, WHO ICTRP, ClinicalTrials.gov 17 July 2017	s-B12: <148 pmol/L, hgb, and MCV, t-Hcy and s-MMA levels.	Comparison between oral vitamin B12 and IM vitamin B12 therapy.

Abbreviations
B12 = vitamin B12, **Hct** = haematocrit, **Hcy** = homocysteine, **hgb** = haemoglobin, **IM** = intramuscular, **MCV** = mean corpuscular value, **MMA** = methylmalonic acid, **NA** = not applicable, **RBC** = red blood cell, **RCT** = randomised controlled trial, **RYGB** = Roux-en-Y gastric bypass, **s** = serum, **t** = total.

Natural course of disease

Five SRs investigating the natural course of vitamin B12 deficiency were included (See ***Table 41***).[32 123-126] The year of publication of the included studies ranged from 2003 to 2016. The number of studies included in each SR ranged from 9 to 57 studies of various study designs, including case reports, observational, case-control, cross sectional, longitudinal, cohort, placebo controlled, meta-analysis, literature review, and cell culture/invitro. The populations of interest included patients with neurodegenerative disease and cognitive impairment,[32] older patients,[123] healthy pregnant women,[125] and women with a prior, or current neural tube defect (NTD)-affected pregnancy.[124 126] In the included studies, the age of patients ranged from 18 years[125] to 99 years;[123] this large spread is due to one study investigating healthy pregnant women and another investigating geriatric patients. Two SRs diagnosed vitamin B12 deficiency using only serum vitamin B12;[32 123] one used serum vitamin B12, MMA, and holoTC;[126] one used serum vitamin B12, MMA, holoTC, Hcy or testing of cord blood;[125] and one used serum or plasma vitamin B12, MMA, holoTC and amniotic fluid B12.[124]

Thirty primary research studies investigating the natural course of vitamin B12 deficiency were also included (see ***Table 44***). The year of publication of the included studies ranged from 1965 to 2019. Six studies were conducted in the USA,[74 127-131] five studies were conducted in the Netherlands,[132-136] three in Canada,[137-139] Denmark,[97 140 141] and France,[142-144] two in both the UK[81 145] and Israel,[146 147] and one each in Cyprus,[148] Czech Republic,[149] Finland,[150] Germany,[151] Ireland[152] and Sweden.[153] All 30 studies were observational in nature with varying study design, including 2 cross-sectional studies, 4 cohort studies, 13 case-control studies and 11 case series. The included patients were mostly older (aged 60 and above) females. One study exclusively evaluated pregnant women and their foetuses/children.

To assess vitamin B12 levels, 15 studies tested only total serum B12,[74 97 129 130 135 139 141 143 145 146 148 149 151-153] 4 studies tested total serum B12 and Hcy,[132 136 142 144] 1 study tested total serum B12 and holoTC,[133] 1 study tested total serum B12 and MMA,[147] 1 study tested total serum B12, methylcitric acid, Hcy and MMA,[137] 1 study tested total serum B12, Hcy and MMA,[131] 1 study tested only holoTC,[138] and 1 study tested only MMA.[140] Five studies did not specify the types of test utilised.[81 127 128 134 150]

6.4 Risk of bias

6.4.1 Systematic reviews

The AMSTAR tool was used to assess the quality of reporting of the included 13 SRs.[52]

Five of the 13 SRs were considered to be of low risk of bias and satisfied more than 70% of AMSTAR criteria.[8 34 119 122 125] Two of these low bias reviews were Cochrane reviews.[8 119] Points were lost in high bias reviews for shortcomings such as the inability to combine study results (not deemed possible in three of the five SRs).[8 119 122] The most common shortcomings included: an unavailable list of excluded studies, a lack of description of use of grey literature, and lack of clarity on an *a priori* design (providing reference to a published protocol and inclusion/exclusion criteria).

Four SRs were graded 'yes' for more than 30%, but less than 70% of the criteria.[118 121 123 126] All SRs included conflict-of-interest information. All four studies had an absent or only partially available list of included and excluded studies. All studies were graded down due to a lack of *a priori* design.[118 121 123 126] Three of four did not employ duplicated study selection and data extraction.[118 121 126]

Four of the thirteen SRs were graded as having a high risk of bias, and satisfied <30% of the criteria. There were several shortcomings such as lacking an *a priori* design, lacking comprehensive literature search, inclusion of grey literature, duplicate study extraction, or quality assessment of studies.[19 32 120 124]

6.4.2 Diagnostic accuracy studies

For the evaluation of diagnostic accuracy from primary studies, Willis (2011)[34] assessed studies using the QUADAS tool; additional primary studies included in the present review were evaluated using QUADAS-2. The tool evaluates risk of bias and applicability of three domains (patient selection, index test(s) and reference standard(s)), and an additional risk of bias domain (flow and timing).

The evidence base included by Willis (2011)[34] was graded poorly against the QUADAS criteria. Unclear spectrum bias was observed across approximately 70% of the evidence base. Verification bias was high in the included studies. Verification bias is introduced when not all samples are tested with the same reference test; 85% and 100% of studies had low differential and incorporation bias, respectively.

Due to a lack of a reference standard for vitamin B12 testing, reference tests were graded as uncertain for both applicability and risk of bias domains of the QUADAS-2 criteria. One study[117] was graded as high-risk in this domain as they utilised a red cell cobalamin test, which currently appears unestablished in the literature. All studies were graded with a low risk of bias in flow and timing. Generally, index tests performed well for risk of bias and applicability. One study[114] was judged down as their study design led to thresholds that were notably different from what is observed in the literature and clinical practice

guidelines. Two studies[113 116] were judged as having a high risk of bias due to not pre-specifying diagnostic thresholds. Evidence quality was mixed for patient selection; three of six studies had uncertain risk of bias due to little clinical data available.[33 114 115] Two studies performed poorly due to a lack of patient data.[113 116] For similar reasons, two of six studies scored uncertain risk in the applicability domain.[113 114] Three additional studies were judged to have a high risk of bias as the recruited samples were from subpopulations (neuropsychiatric[115] or elderly[117] patients). One study had a high level of vegetarian and vegan participants as they were recruited from a natural medicine-oriented clinic.[116]

The QUADAS-2 tool evaluation of the six diagnostic accuracy studies have been presented in ***Table 47, Appendix C.***

6.4.3 Change in management studies

A quality appraisal checklist for case series studies developed by IHE was used to evaluate the risk of bias in the change in management studies, which were cross sectional by design. For further details pertaining to this evaluation, see ***Table 48***, ***Appendix C***. Items 7, 9, 13, 16, and 18 were considered not applicable (NA) in the evaluation of the included studies.

Across the evidence base, all studies satisfied 9 of the 15 items (***Table 48***). These were items 1, 5, 6, 8, 10, 14, 15, 17, and 19. No study explicitly reported blinding of outcome assessors or reported both competing interests and sources of support.

Carmel (1986) was judged as having a low risk of bias against the IHE checklist.[58] The study was judged down due to recruiting in a single centre, not blinding, and not reporting on conflict of interest. Hvas (2000) was also judged as low risk of bias. Both Hvas (2000) and Hvas (2002) were retrospective and unblinded.[59 60] Although the outcomes were subjectively measured, the studies used specific criteria and alternative objective measures were not available. Consequently, item 12 was considered only partially satisfied for both studies.[59 60] Conflicts of interest were not reported.

6.4.4 Natural course of disease studies

The quality of single-arm trials investigating the natural course of vitamin B12 deficiency was appraised using the IHE quality appraisal checklist, and the quality of case-control and cohort trials was appraised using the SIGN quality appraisal tools. Cross-sectional studies were evaluated using the case-control tool, noting the applicability of certain domains was uncertain. Thus, the assessment of bias of these studies is limited. Summaries of the risk of bias are presented in ***Table 49*** to ***Table 51***. For the assessment of single-arm trials, the IHE checklist was modified to better assess the natural course of disease studies. Questions relating to “whether the intervention was clearly described” was amended to “whether the method of diagnosis was clearly described.” Further, the question relating to adverse events was not applicable to the appraised study design, given interventions were not frequently utilised.

The quality and bias issues were similar across the case-control and single-arm trials. Therefore, both study designs are discussed together. Overall, the studies were of moderate quality. Studies generally stated their objectives, described the diagnostic thresholds and co-interventions appropriately, defined outcomes *a priori* and used suitable methods to assess each outcome. The conclusions were generally consistent with the results.

All cohort studies appropriately and clearly addressed the aim with clearly defined outcomes and used a reliable method of measuring exposure. No study assessed or accounted for the likelihood that the subjects may have the outcome at the time of enrolment, made comparisons between full participants and those lost to follow up by exposure status, and blinding was not performed or accounted for. These studies (k = 4) were judged as moderate to high risk of bias.

Key limitations related to the under-reporting of certain methodological domains and the lack of appropriate statistical tests. For example, in several trials it was not reported whether studies were prospectively or retrospectively performed or whether patients were recruited consecutively. The eligibility criteria were often limited to inclusion criteria, with few studies reporting exclusion criteria, an effect more pronounced in older publications. Most trials reported a study characteristics table; however, the variables presented were often limited to age, sex and vitamin status. It was unclear whether patients were at a similar point in the disease because comorbidities and other concurrent diseases were not reported and vitamin B12 levels did not correlate with symptom severity.[16] Further, conflicts of interest were partially (limited to source of funding) or not stated and it was not reported whether the outcome assessors were blinded to disease status or treatments. The lack of blinding was not a substantive issue for objective outcomes such as vitamin status. However, for outcomes involving judgement (e.g. depression scores), knowledge of the disease status could potentially influence reporting.

The appropriateness of the statistical analysis varied across the included trials. Several trials attempted to correct for potential confounding variables (e.g. age and education level) using multivariate analysis, whereas others did not. These studies performed basic statistical comparisons or described the results narratively. Lastly, the lack of power calculations (in most trials) and the absence of appropriate statistical tests, raised uncertainties as to how generalisable the results are to the broader intended population.

6.5 Applicability of evidence base to Switzerland

The assessment of applicability aims to determine how generalisable the studies included in the HTA are to the Swiss context. It involves the comparison of demographic and clinical characteristics in the included trials to Swiss practice. There was limited published literature reporting the demographics of, and clinical characteristics associated with, vitamin B12 deficiency in Switzerland. An overview of the Swiss clinical characteristics are provided in ***Table 13***.

Table 13 Characteristics of vitamin B12 testing in Switzerland

Parameter	Characteristics
Demographics	Age: older adults (>55 years)[154] Sex: equal % of males and females to mostly female[30 154] Comorbidities: unknown % of patients with renal failure: unknown
Vitamin B12 deficiency thresholds	Risch 2015 (healthy patients aged 60+)[30] *Probable deficiency* • B12: <131 pmol/L • MMA: >0.49 µmol/L • HoloTC: <25.8 pmol/L *Grey zone* • B12: 131-315 pmol/L • MMA: 0.21-0.49 µmol/L • HoloTC: <25.8-56.9 pmol/L MediX guideline[38] *Deficiency* • B12: <150 pmol/L • MMA: >0.27 µmol /L • HoloTC – NA *Grey zone* • B12: 150-250 pmol/L
Treatment	Treatment for deficiency[38] • IM or subcutaneous 1,000 µg of cyanocobalamin or hydroxocobalamin on day 1, 3 and 5 or days 1-5, then once per week for 1 month and then every 1-3 months thereafter Oral therapy also available (1,000 µg)
Settings	General practitioner, endocrinologist, haematologist Primary care setting or hospital

Abbreviations
B12 = vitamin B12, **holoTC** = holotranscobalamin II, **IM** = intramuscular, **MMA** = methylmalonic acid, **NA** = not applicable, **pmol/L** = picomoles per litre., **µmol/L** = micromoles per litre, **µg** = micrograms.

6.5.1 Diagnostic accuracy

The studies included in Willis (2011)[34] were from Asia, Europe, and North America, with most patients from the UK and USA. It is unclear to what extent the included patients were reflective of Swiss patients with B12 deficiency owing to an absence of published literature evaluating vitamin deficiency in Switzerland. This has important implications when considering the applicability of the results because the accuracy of vitamin B12 tests are dependent on specific demographic factors, such as the presence of renal insufficiency and the age of the patient. Without knowing these demographic factors in Switzerland, it is unclear how applicable the results are.

The thresholds for vitamin deficiency (using serum B12) used by the trials included in Willis (2011)[34] were broadly consistent with those listed in the MediX guidelines[38] and those calculated by Risch

(2015).[30] The thresholds used to determine B12 deficiency using MMA were variable in Willis (2011);[34] however, they fell within the range reported in the Swiss studies. Importantly, it was unclear to what extent the MediX guideline is utilised in, and is representative of, contemporary Swiss practice. Likewise, it was uncertain whether the patients included in Risch (2015)[30] are reflective of the general Swiss population who may be tested for vitamin B12 deficiency.

6.5.2 Change in management

The change in management studies were performed in Denmark in the 2000's[59 60] and USA in the 1980's.[58] It is unclear to what extent these studies are applicable to the Swiss context given the absence of information specific to Switzerland. Additionally, there were likely regional and temporal differences in practice that exist between the included studies and contemporary Swiss practice.

The thresholds for serum B12 deficiency were higher in the included trials compared to the MediX guideline[38] and the study by Risch (2015).[30] The MMA cut-off in Carmel (1986)[58] and Hvas (2000 and 2002)[59 60] were above those in MediX guideline[38] but not Risch (2015).[30] However, the revised MMA cut-off in Hvas (2000 and 2002)[59 60] was similar to current Swiss guidelines.

6.5.3 Effectiveness of change in management

The applicability of the change in management studies are uncertain. The trials include diverse populations with varying indications and causes of vitamin B12 deficiency. It is unclear to what extent these match the demographic profile of patients with vitamin B12 deficiency in Switzerland. The reference levels for B12 deficiency using serum vitamin B12 are similar in the primary studies and the MediX guidelines.[38] However, it was unclear whether the SRs evaluating the natural course of disease align with the guidelines as the cut-off values varied. The SRs evaluating supplementation are broadly congruent to the MediX guidelines,[38] both recommend oral and IM supplementation. However, the frequency of dosing, preferred route of administration and indication differ.

6.6 Results: Direct evidence of clinical effectiveness

No direct evidence on the effectiveness of vitamin B12 testing was identified. Therefore, a linked evidence analysis was conducted.

6.7 Results: Linked evidence of clinical effectiveness

Based on the available literature, it was not possible to separate the results of included studies into the two key populations under investigation in this review. Therefore, the results presented in this section are predominantly reflective of a broad population of patients tested for suspected B12 deficiency.

6.7.1 Diagnostic accuracy

A single SR (including meta-analysis) was identified that reviewed the diagnostic accuracy of serum vitamin B12 tests against reference tests MMA, holoTC, Hcy, and clinical diagnosis.[34] Clinical reference refers to response to treatment. No SRs were identified that evaluated the diagnostic accuracy of MMA or holoTC as an index test.

Among all subgroups, the meta-analysis reported that the serum vitamin B12 test had a positive likelihood ratio close to one. This suggests the test is inefficient in differentiating between deficient and healthy patients. Low sensitivity was determined, with variable specificity (***Table 14***). These findings were highly heterogeneous. The meta-analysis demonstrated low sensitivity and variable specificity among all reference methods and subgroups. The estimated sensitivity was higher in two-gated studies compared to single-gated studies, and specificity was highest in single-gated studies with unknown vitamin B12 status. Sensitivity was lowest when Hcy was used as a reference test (***Table 14***). Average specificity was relatively similar across reference tests; however, the range was highly variable (0.36–0.96). The variability of specificity was still high (0.68–0.89) when considering only MMA and holoTC as reference tests, which are the focus of this short report. Thresholds were variable across the studies included in the Willis (2011) review.[34] These are summarised in ***Appendix B***, ***Table 45.***

Table 14 Sensitivity and specificity of serum vitamin B12 testing against reference tests MMA, Hcy, holoTC, and clinical reference[34]

Reference standard	Number of studies (number of patients)	Sensitivity (95% CI)	Specificity (95% CI)
MMA	18 (NR)	0.52 (0.39-0.65)	0.81(0.70-0.89)
Hcy	18 (NR)	0.40 (0.27-0.54)	0.84 (0.73-0.90)
HoloTC	4 (NR)	0.70 (0.55-0.82)	0.79 (0.68-0.86)
Clinical reference	6 (NR)	0.73 (0.38-0.92)	0.78 (0.36-0.96)

Abbreviations
CI = confidence interval, **Hcy** = homocysteine, **HoloTC** = holotranscobalamin II, **MMA** = methylmalonic acid, **NR** = not reported.
Source
Willis 2011[34]

Using Hcy and MMA as reference tests, studies measuring vitamin B12 with a chemiluminescent assay performed with significantly lower sensitivity than studies performing radioassays (most commonly used). Although not a focus, findings by Willis (2011)[34] suggest that age does not influence test sensitivity or specificity (contrary to previous suggestions by others).[155-157] Furthermore, they reported no influence of clinical indication or assay type on diagnostic performance. Studies that enrolled healthy controls reported higher sensitivities (possibly impacted by selection bias) than single-gated studies that exclusively enrolled patients with an unknown vitamin B12 status. Studies performed in general practice had low sensitivity (MMA 0.43; Hcy 0.24).

Notably, these findings were affected by the quality and limitations of the evidence base. Imperfect reference standards pose a challenge to the evaluation of diagnostic accuracy.

Primary research published after 2009 was included to supplement the findings of the Willis (2011) review (k = 6).[33 113-117]

Test specificity and sensitivity is greatly influenced by the selected cut-off value. ***Table 15*** demonstrates the broad thresholds used across the primary studies and the variability of sensitivity and specificity reported. The primary studies (k = 6) found the sensitivity and specificity of the tests was generally poor when the threshold was similar to current guidelines.

Table 15 Primary studies investigating the diagnostic accuracy of vitamin B12 deficiency testing

Author, year	Study design; Risk of bias (QUADAS-II)	Population, n, % affected individuals	Reference test	Index test	Sensitivity	Specificity	Threshold
Campos, 2020[A33]	Retrospective paired two-gate diagnostic test accuracy study At risk of bias	Mixed patient population assessed 4cB12 model (n = 11,833), 8.7% participants with low or deficient B12 (determined by all tests)	4cB12 model Threshold (subclinical/possibly deficient): ≤ -0.5 and > -1.5	B12	86.1%	77.7%	<229 pmol/L
				HoloTC	85.7%	81.2%	<45 pmol/L
				MMA	81.8%	83.4%	>0.25 µmol/L
			4cB12 model Threshold (probable deficiency): ≤ -1.5	B12	94.8%	92.3%	<167 pmol/L
				HoloTC	93.1%	96.0%	<27 pmol/L
				MMA	94.8%	96.4%	>0.47 µmol/L
Heil, 2012[113]	Prospective, paired two-gate diagnostic test accuracy At risk of bias	Patients with suspected vitamin B12 deficiency (n = 360), 23.9% participants had below cut-off (determined by serum B12 test)	MMA Threshold: >0.45 µmol/L	B12	53%	81%	<145 pmol/L
				HoloTC	64%	88%	<21 pmol/L
Herrmann, 2013[114]	Prospective, paired single-gate diagnostic test accuracy At risk of bias	Samples sent for total vitamin B12 assay (n = 1,359), 18.6% of participants had elevated MMA with normal creatinine	MMA Threshold: >0.30 µmol/L	B12	72%	41%	<227 pmol/L
				HoloTC	72%	54%	<35 pmol/L

Author, year	Study design; Risk of bias (QUADAS-II)	Population, n, % affected individuals	Reference test	Index test	Sensitivity	Specificity	Threshold
Schrempf, 2011[115]	Retrospective, paired single-gate diagnostic test accuracy At risk of bias	Patients with neurological symptom from vitamin B12 deficiency (n = 1,279), 69% in grey zone (determined by holoTC)	MMA Threshold: >0.298 µmol/L	B12	66.2%	62.1%	<206 pmol/L
				HoloTC	56.3%	50.5%	<42 pmol/L
Schwarz, 2015[A116]	Prospective, paired single-gate diagnostic test accuracy At risk of bias	Samples sent from a natural-medicine oriented practice (n = 77), 17.3% (determined by holoTC and MMA)	HoloTC Threshold: <50 pmol/L (subclinical/possibly deficient)	B12 (CLIA)	38%	94%	<156 pmol/L
				B12 (MTP)	50%	96%	<212 pmol/L
				MMA	40%	94%	>0.30 µmol/L
			HoloTC Threshold: <35 pmol/L (probably deficient)	B12 (CLIA)	53%	93%	<156 pmol/L
				B12 (MTP)	71%	95%	<212 pmol/L
				MMA	41%	90%	>0.30 µmol/L
Valente, 2011[117]	Prospective paired single-gate diagnostic test accuracy study At risk of bias	Elderly patients (n = 700), 9.6% (determined by red cell cobalamin) or 8.0% (determined by serum B12)	Red cell cobalamin Threshold: <33 pmol/L	B12	33%	95%	<123 pmol/L
				HoloTC	55%	96%	<20 pmol/L
				MMA	81%	63%	>0.36 µmol/L

Abbreviations
B12 = serum or plasma vitamin B12, **CLIA** = chemiluminescent immunoassay, **MTP** = microbiological tests with microtitre plates, **holoTC** = holotranscobalamin II, **MMA** = methylmalonic acid.
Notes
[A] Results are presented from a subpopulation with a "possible" or "probable" B12 deficiency.

6.7.2 Change in management

Three change in management studies were identified.[58-60] The studies addressed the role of vitamin B12 testing in clinical decision-making regarding the commencement of vitamin B12 supplementation. These comprised two groups; Hvas (2000 and 2002)[59 60] investigated management decisions in relation to plasma MMA test results; Carmel (1986)[58] investigated management decisions to serum vitamin B12

test results. ***Table 43*** (***Appendix B***) provides a summary of results for the included change in management studies.

Hvas (2000)[59] addressed how plasma MMA test results were used during treatment decision-making in Denmark district hospitals. Plasma B12 cut-off values ranged from 200–600 pmol/L in healthy patients. Plasma MMA laboratory reference ranges were 0.05–0.37 µmol/L until 30 June 1996 and 0.08–0.28 µmol/L thereafter. To account for renal insufficiency, plasma creatinine levels were also measured. The reference interval used was 55–120 µmol/L. Medical records of patients with plasma MMA measurements above the plasma MMA laboratory cut-off values (n = 177) were obtained from 3 hospitals between 1995 and 1996. Plasma MMA was measured by gas chromatography mass spectrometry.

Hvas (2000)[59] found that in 177 patients, no action was taken in 62% of cases based on the MMA test result (median plasma MMA = 0.46 µmol/L; this is an elevated value indicative of deficiency). A vitamin B12 deficiency was diagnosed in 22% of cases. In 12% of patients, some action was taken without a diagnosis. If plasma MMA was above 0.99 µmol/L (well above the laboratory established reference values), 68% of physicians responded. There was also a response in 32% of patients with low plasma MMA. There was no tendency to react when plasma MMA was below 1.00 µmol/L.

Plasma vitamin B12 was measured in 72% of patients. Despite returning normal plasma vitamin B12 results (61%), 18% of patients were diagnosed with a vitamin B12 deficiency. Six of 177 (3%) patients were diagnosed as non-deficient, irrespective of their results presenting a strong indication of deficiency, including plasma MMA >0.99 µmol/L and plasma vitamin B12 <200 pmol/L.

Contrary to general recommendations, physicians were using plasma MMA as a primary diagnostic test. Furthermore, the plasma MMA test was ordered simultaneously with the plasma vitamin B12 test. Plasma vitamin B12 was only determined prior to a plasma MMA test in 10% of cases. The treatment pathway after MMA testing in the district hospital is depicted in ***Figure 4***. This demonstrated—in the author's judgement—the extent to which diagnosed deficient patients were not followed-up for further testing. Further examinations refer to testing of plasma homocysteine, antibodies against parietal cells or IF, schilling test or dicopac test, upper gastrointestinal endoscopy, or bone marrow testing. The authors judgement was based on current recommendations incorporating test results of elevated plasma MMA (until June 30 1996, >0.37 µmol/L, >0.28 µmol/L onwards), very high MMA (>0.99 µmol/L), and low serum B12 (<200 pmol/L).

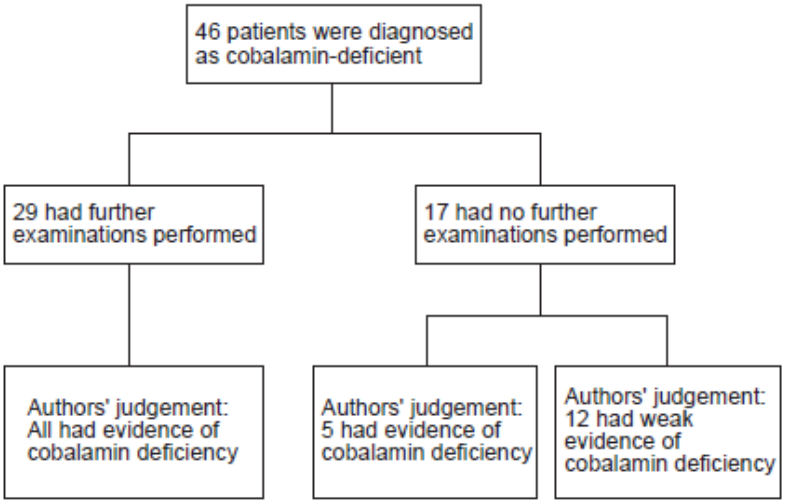

Figure 4 Pathway of vitamin B12 deficient individuals in three Danish district hospitals[59]

Source
Hvas 2000[59]

It is notable that this study only investigated three hospitals and may not necessarily reflect the clinical practice across all Danish hospitals. Hvas (2000)[59] noted the lack of continuity between physician and patient may contribute to inconsistent management decisions and failure to respond to increased plasma MMA. Doctors requesting tests were often different to the doctors making the evaluation.

The same group (Hvas 2000)[59] published a second study in 2002 examining the use of plasma MMA in general practice between 1997 and 1998.[60] The study included 181 patients with MMA >0.28 µmol/L that were registered at any one of 10 practices in Aarhus County. The group searched for reactions under the same conditions as the study previously described.[59] There was no reaction from general practitioners (GPs) in 29% of cases. A reaction was reported in the remaining 71% of patients. In 56% of cases vitamin B12 therapy was commenced (median MMA = 0.51 µmol/L) and MMA testing was repeated in 19 patients. There was a significant association between a reaction from a GP and plasma MMA. Unlike the findings by Hvas (2000), in a hospital setting the GPs generally followed current recommendations for beginning vitamin B12 therapy in 84% of patients with plasma MMA above the cut-off (0.44 µmol/L). Often plasma MMA was used for screening. It was suggested that patients with only slightly increased plasma MMA were over-treated.

Carmel (1986)[58] investigated 250 patients with low serum vitamin B12 levels. Patients were grouped into three categories. Group one included patients who received adequate management (adequate investigation ruling in or out a deficiency, further workup to determine cause of deficiency, and vitamin B12 therapy begun in diagnosed individuals); 34% of patients belonged to this group. Group two comprised 42% of cases where no physician action was taken after a low serum vitamin B12 measurement was obtained. To fit the criteria of ‘no response’, no further diagnostic testing was

performed, no supplementation commenced, and failure to note low levels in daily notes or discharge forms. The third group contained 24% of the cohort. These were individuals who received suboptimal management. This was defined as individuals with low serum B12, and who received inadequate or no work up. Individuals in this group sometimes received supplementation without evidence that it was required. Supplements were usually administered orally regardless of the disease aetiology. In 10 patients a single parenteral injection was administered without follow-up or long-term supplementation appointments. Eight patients received no therapy at all despite a low vitamin B12 measurement. This study did not consider renal insufficiency or creatinine values, and was published prior to the mainstream use of reference tests.

The results from Carmel (1986)[58] suggest that patients were not managed appropriately at this medical centre, and support findings from the Danish district hospitals assessed by Hvas 2000.[59] However, Hvas (2000)[59] and Carmel (1986)[58] reflect management in 1995–1996 and 1983–1984, respectively. The applicability of these findings to current practice is questionable and the limitations of these results should be considered.

Hvas (2000)[59] was performed shortly after plasma MMA testing became common practice. Over the following two decades since the publication of this study, it is possible a more widespread understanding of MMA testing allows for management decisions in-line with clinical recommendations.

6.7.3 Effectiveness of change in management

Supplementation (false positive test results)

Patients that receive a positive test result will be subject to B12 supplementation. In false positive cases, these patients will be subject to any potential safety issues associated with this treatment, with none of the potential benefits. Potential safety issues associated with vitamin B12 supplementation are discussed in ***Section 6.8***.

Supplementation (true positive test results)

Patients that receive a true positive test result will be subject to supplementation therapy, in which case the benefits and harms of B12 supplementation should be considered.

Eight SRs were identified relating to vitamin B12 supplementation in people at risk of, or with a clinical suspicion of, vitamin B12 deficiency.[19 32 118-122 126] The studies investigated the differences between dosage and IM and orally administered vitamin B12 supplements. In some studies, this was performed comparatively to no treatment or placebo. In participants (of varying populations) with deficient/low

vitamin B12 levels, high doses of orally administered vitamin B12 was found to be as effective as IM administration in correcting vitamin B12 deficiency,[8 122] prophylaxis in bariatric surgery patients,[120] and treating pernicious anaemia.[19] High doses of oral vitamin B12 was also found to be as effective as IM administration in achieving clinical,[118] neurological,[119] and haematological responses;[119] however, it was found that caution should be taken when supplementing oral vitamin B12 in patients with severe neurological manifestations as IM supplementation is the preferred method to elicit a rapid response.[118] In post-bariatric and Roux-en-Y surgery patients, there was contrasting evidence between two of the reviews. Mawahar (2018)[120] suggested that an oral dosage of 1,000 µg may be appropriate to prevent the development of vitamin B12 deficiency. Smelt (2017)[122] suggested that 350 µg (orally administered) was sufficient. In older patients with and without vitamin B12 deficiency, vitamin B12 supplementation had no effect on haematocrit, MCV and RBC levels.[121]

Table 16 provides a summary of study characteristics for the included SRs on the treatment effectiveness of vitamin B12 supplementation.

Table 16 Results of systematic reviews on the treatment effectiveness of vitamin B12 supplementation

Author, year	Indication; comparison; number of included studies	Findings	Risk of bias (AMSTAR)
Andres 2018[118]	Patients with vitamin B12 deficiency due to gastrointestinal disorders; oral vs. IM vitamin B12, nasal vitamin B12; k = 39	Oral vitamin B12 is an effective alternative to IM injections of B12 in restoring serum B12 levels and clinical manifestations, except in patients with severe neurological manifestations. Oral B12 avoids the discomfort, contraindications (in patients with anticoagulation) and cost of monthly injections.	Moderate
Butler 2006[119] (distillation of a Cochrane review)	Patients with low serum B12; oral vs. IM vitamin B12; k = 2	Oral vitamin B12 (1000 and 2000 µg) may be as effective as IM injections of B12 with respect to haematological and neurological responses.	Low
Chan 2016[19]	Patients with pernicious anaemia; oral vs. IM vitamin B12; k = 12	Oral vitamin B12 is an effective alternative to IM injections of vitamin B12 for the treatment of vitamin B12 deficiency.	High
Mahawar 2018[120]	Patients who have undergone RYGB; oral vitamin B12; k = 19	To prevent vitamin B12 deficiency following RYGB, a dose of 1000 µg of vitamin B12 (oral) daily is adequate. Doses below 15 µg of vitamin B12 were insufficient for prophylaxis.	High
Moore 2012[32]	Patients with cognitive impairment and/or dementia; oral and parenteral vitamin B12; k = 43	Vitamin B12 supplementation corrected vitamin B12 deficiency in patients with dementia. However, supplementation only improve cognitive performance in a small sub-set of patients.	High
Smelt 2017[122]	Patients who have undergone bariatric surgery; oral and IM injections of vitamin B12; k = 10	To treat low vitamin B12 levels following bariatric surgery, 350 µg of vitamin B12 (oral) is recommended.	Moderate
Smelt 2018[121]	Older adults with or without vitamin B12 deficiency; oral and IM injections of vitamin	No impact of vitamin B12 supplementation on haematological outcomes in patients with and without vitamin B12 deficiency.	Moderate

Author, year	Indication; comparison; number of included studies	Findings	Risk of bias (AMSTAR)
	B12; k = 4		
Wang 2018[8]	Patients with vitamin B12 deficiency; oral vs. IM injections of vitamin B12; k = 3	Oral vitamin B12 was as effective as IM injections of B12 with respect to normalisation of serum B12 levels in patients with deficiency. However, oral treatment was cheaper.	Low

Abbreviations
B12 = vitamin B12, **k** = number of studies; **IM** = intramuscular, **mg** = milligrams, **RCTs** = randomised controlled trials, **RYGB** = Roux-en-Y gastric bypass, **µg** = micrograms.

No supplementation (true negatives)

Patients that receive a true negative result will avoid unnecessary supplementation.

Symptomatic individuals may be followed up 1-2 months after presentation to confirm their vitamin B12 status (See the 'test' pathway in ***Figure 1***). The prevalence of negative individuals in samples from patients with suspected vitamin B12 deficiency is high (81.4% with normal MMA,[114] 90.4% as determined by the 4cB12 algorithm (incorporating serum B12, holoTC, MMA, and homocysteine test results).[33] Without testing, these symptomatic individuals will likely undergo the 'no test' pathway as depicted in ***Figure 1***.

Natural course of the disease (false negatives)

Patients that receive a false negative test result will be subject to the natural, untreated course of the disease.

Five SRs that investigated the natural course of vitamin B12 deficiency were included.[32 123-126] Studies found that insufficient vitamin B12 levels left untreated can lead to conditions ranging from mild to severe. Insufficient vitamin B12 levels were found to be associated with Alzheimer's disease, vascular dementia, Parkinson's disease and cognitive impairment,[32] depression,[123] and NTDs.[124 126] An association was found between vitamin B12 insufficiencies and low birth weight, however, much of the effect was attributable to studies from India and may not be applicable to a broader population.[125]

Additionally, 30 primary research studies that investigated the natural course of vitamin B12 deficiency were also included.[74 81 97 127-153] Studies reported vitamin B12 deficiency when left untreated can lead to conditions ranging from mild to severe. However, several studies also reported a lack of association between B12 and symptoms. In adults vitamin B12 deficiency was associated with the development of neurological symptoms and disorders (i.e., polyneuropathy, peripheral nerve dysfunction, paraesthesia, ataxia, dementia, sensory deficits),[127 129 130 134 141 143 147] psychological disorders (i.e., depression),[131 133]

cognitive impairment,[74 81 133 137 153] haematological abnormalities (i.e., anaemia, thrombosis),[142 143] increased cardiovascular risk factors,[136 148] oral and cutaneous symptoms,[143 145] gastritis,[150] dyspnoea,[128] weight loss,[128] and pain.[74] Maternal B12 deficiency has been linked to developmental delay,[149] failure to thrive,[149] hypotonia,[149] anaemia,[149] microcephaly,[149] increased heart rate in their children,[135] and increased risk of NTDs,[138 152] very early recurrent abortion,[144] and infertility/foetal loss.[146]

Table 17 and ***Table 44*** (***Appendix C***) provide a summary of study characteristics for the included SRs and primary research studies on the natural course of vitamin B12 deficiency.

Table 17 Effectiveness of change in management: Natural course of B12 deficiency (systematic reviews)

Study; country	Indication; aim of study; number of included studies	Databases searched and search date	Vitamin B12 deficiency criteria	Findings	Risk of bias (AMSTAR)
Moore 2012[32] Australia	Patients with cognitive impairment or dementia; determine if there is an association between vitamin B12 levels and cognitive impairment or dementia; k = 43	Medline, PubMed, PsychINFO March 2011	Deficient s-B12: <150 pmol/L Low-normal s-B12: 150-250 pmol/L	Low-normal of B12 levels associated with Alzheimer's disease, vascular dementia & Parkinson's disease. B12 deficiency associated with cognitive impairment.	High
Petridou 2016[123] Greece	Older adults: determine if there is an association between serum B12 with depression in the elderly; k = 9	Medline June 2013	Low s-B12 ranged from <170 pmol/L to <364 pmol/L	Low serum B12 levels was associated with depression in older adults. The greatest association between B12 and depression was observed in females.	Moderate
Ray 2003[124] Canada	Women with previous or current NTD-affected pregnancy and unaffected controls; to determine if there is an association between maternal B12 levels and risk of NTD; k = 17	Medline October 2002	NR	Low maternal B12 levels were associated with an increased risk of developing NTD during pregnancy.	High
Sukumar 2016[125] UK	Pregnant women; to determine if there is an association between maternal B12 levels and birth weight; k = 23	Medline/ PubMed, Embase, Global Health, Commonwealth Agricultural Bureau & CINAHL December 2014	NR	Association between low maternal B12 levels and birth weight. Sub-group analyses noted the effect may be limited to studies from India.	Low

Study; country	Indication; aim of study; number of included studies	Databases searched and search date	Vitamin B12 deficiency criteria	Findings	Risk of bias (AMSTAR)
Wang 2012[126] China	Women with previous or current NTD-affected pregnancy and unaffected controls; to determine if there is an association between maternal B12 levels and risk of NTD; k = 9	PubMed, Medline, Elsevier Science & Springer Link, China National Knowledge Infrastructure, Vip information & China biology medical literature database	Cut-off varied across studies	Low maternal B12 levels were associated with an increased risk of developing NTDs.	Moderate

Abbreviations
B12 = vitamin B12, **CINAHL** = Cumulative Index to Nursing and Allied Health Literature, **Hcy** = homocysteine, **holoTC** = holotranscobalamin II, **MMA** = methylmalonic acid, **NR** = not reported, **NTD** = neural tube defects, **p** = plasma, **pmol/L** = picomoles per litre, **s** = serum, **UK** = United Kingdom.

6.8 Results: Safety

Testing for vitamin B12 deficiency requires venepuncture (blood draw). This is a safe and commonly performed procedure.

The main risks associated with the vitamin B12 tests are related to their low diagnostic accuracy, leading to false negative and false positive results.

Vitamin B12 supplementation may be administered by oral or IM routes. These are safe and commonly used routes for administration of medication.[119] Due to the water-soluble nature of vitamin B12 supplements (in the form of hydroxocobalamin or cyanocobalamin), overdose or over-prescription is considered highly unlikely.[158] The reported side effects of hydroxocobalamin and cyanocobalamin are rare, mild and temporary.[159 160] Rarely, cases of anaphylaxis after supplementation therapy have been reported.[161 162] Evidence from the identified five systematic reviews suggest that oral and IM therapies have equal safety.[8 119] Consequently, safety concerns are low, even in the case of a false positive result. The natural progression of a vitamin B12 deficiency can lead to severe and permanent disease (see ***Section 6.7.3***). Therefore, there are safety concerns regarding false negative results in which the patient remains untreated and the disease may progress.

6.9 GRADE summary of findings

The following tables (***Table 18***,***Table 19*** and ***Table 20***) summarise the overall strength of evidence supporting the key findings related to the diagnostic accuracy of the three index tests under investigation. Outcomes related to the impact of B12 testing on management decisions are not summarised in the GRADE tables due to the absence of contemporary evidence relevant to the research question. Similarly, the effectiveness of these management decisions (i.e. on true positive, true negative,

false positive, and false negative patients) are not reported in the GRADE tables, as they are not the main focus of the review.

Evidence from primary studies of total B12 that was summarised in the report narratively is not represented in the GRADE tables, due to the risk of misinterpreting unweighted ranges of reported values in contrast to the results of the meta-analysis results reported by Willis (2011).[34] Instead, only the results of the Willis (2011)[34] meta-analysis are reported in the summary of findings table for this index test.

For the remaining index tests (i.e. holoTC and MMA), it was not appropriate to report ranges of accuracy across the included primary studies because studies used difference reference standards. Instead, the results from Campos (2020) are summarised in the summary of findings tables, as this study included the largest sample size with the most generalisable patient population (i.e. conducted in Switzerland).[33]

Swiss-specific prevalence estimates were used to calculate the estimates for the number of results (i.e. true positives, true negative, false positives, false negatives) per 1,000 tested patients, noting that these estimates are only relevant to a general population above the age of 60.[30] The estimated prevalence of B12 deficiency in other subgroups of the target population defined in the PICO (i.e. patients with suspected, or high risk of, B12 deficiency), is unknown. In addition, there are no summary estimates of sensitivity and specificity for these subgroups. Consequently, the absolute effects could not be expressed in the summary of findings table.

The certainty of evidence supporting an outcome, as scored according to the GRADE approach, is defined into the following categories:[57]

- **High certainty**: We are very confident that the true effect lies close to that of the estimate of the effect.
- **Moderate certainty**: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
- **Low certainty**: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect.
- **Very low certainty**: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect.

Table 18 GRADE summary of findings - serum B12

Patient or population: Patients with a suspected, or high risk of, B12 deficiency
Setting: General practice, hospital outpatient, hospital inpatient
New test: Serum B12 | **Cut-off value**: NR*
Reference test: MMA | **Threshold**: NR*
Pooled sensitivity: 0.52 (95% CI: 0.39 to 0.65) | **Pooled specificity**: 0.81 (95% CI: 0.70 to 0.89)

Test result	Number of results per 1,000 patients tested (95% CI)			Number of participants (studies)	Certainty of the Evidence (GRADE)
	Prevalence 8% Typically seen in a general Swiss population aged 60-69	Prevalence 13% Typically seen in a general Swiss population aged 70 to 79	Prevalence 19% Typically seen in a general Swiss population aged 80 and older		
True positives	42 (31 to 52)	68 (51 to 85)	99 (74 to 124)	NR* (18)	⨁◯◯◯** VERY LOW
False negatives	38 (28 to 49)	62 (45 to 79)	91 (66 to 116)		
True negatives	745 (644 to 819)	705 (609 to 774)	656 (567 to 721)	NR* (18)	⨁◯◯◯** VERY LOW
False positives	175 (101 to 276)	165 (96 to 261)	154 (89 to 243)		

Abbreviations
B12 = vitamin B12, **CI** = confidence interval, **MMA** = methylmalonic acid, **NR** = not reported.
Notes
* Not reported in Willis (2011).[34]
** Evidence was graded down due to high risk of bias, applicability concerns, and heterogeneity in reported estimates.
GRADE Working Group grades of evidence[57]
High certainty: We are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect.
Very low certainty: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect.

Table 19 GRADE summary of findings - holoTC

Patient or population: Patients with a suspected, or high risk, of B12 deficiency
Setting: Unknown
New test: holoTC | **Cut-off value**: <45 pmol/L
Reference test: 4cB12 | **Threshold**: ≤-0.5 to >-1.5
Single study sensitivity: 0.86 (95% CI: NR) | **Single study specificity**: 0.81 (95% CI: NR)

Test result	Number of results per 1,000 patients tested (95% CI)			Number of samples (studies)	Certainty of the Evidence (GRADE)
	Prevalence 8% Typically seen in a general Swiss population aged 60 to 69	Prevalence 13.4% Typically seen in a general Swiss population aged 70 to 79	Prevalence 19% Typically seen in a general Swiss population aged 80 and older		
True positives	69 (NR)	115 (NR)	163 (NR)	11,833 (1)	⨁⨁◯◯* LOW
False negatives	11 (NR)	19 (NR)	27 (NR)		
True negatives	747 (NR)	703 (NR)	658 (NR)	11,833 (1)	⨁⨁◯◯* LOW
False positives	173 (NR)	163 (NR)	152 (NR)		

Abbreviations
B12 = vitamin B12, **CI** = confidence interval, **HoloTC** = holotranscobalamin II, **NR** = not reported, **pmol/L** = picomoles per litre.
Notes
* Evidence was graded down due to high risk of bias, and heterogeneity in reported estimates.
GRADE Working Group grades of evidence[57]
High certainty: We are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect.
Very low certainty: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect.

Table 20 GRADE summary of findings - MMA

Patient or population: Patients with a suspected, or high risk, of B12 deficiency
Setting: Unknown
New test: MMA | **Cut-off value**: >0.25 µmol/L
Reference test: 4cB12 | **Threshold** : ≤-0.5 to >-1.5
Single study sensitivity: 0.86 (95% CI: NR) | **Single study specificity**: 0.81 (95% CI: NR)

Test result	Number of results per 1,000 patients tested (95% CI)			Number of participants (studies)	Certainty of the Evidence (GRADE)
	Prevalence 8% Typically seen in a general Swiss population aged 60 to 69	Prevalence 13.4% Typically seen in a general Swiss population aged 70 to 79	Prevalence 19% Typically seen in a general Swiss population aged 80 and older		
True positives	65 (NR)	106 (NR)	155 (NR)	11,833 (1)	⨁⨁◯◯* LOW
False negatives	15 (NR)	24 (NR)	35 (NR)		
True negatives	767 (NR)	726 (NR)	676 (NR)	11,833 (1)	⨁⨁◯◯* LOW
False positives	153 (NR)	144 (NR)	134 (NR)		

Abbreviations
B12 = vitamin B12, **CI** = confidence interval, **MMA** = methylmalonic acid, **NR** = not reported, **µmol/L** = micromoles per litre.
Notes
* Evidence was graded down due to high risk of bias, and applicability concerns.
GRADE Working Group grades of evidence[57]
High certainty: We are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect.
Very low certainty: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect.

7 Legal, social, ethical issues

7.1 Summary statement legal issues

The literature addressing legal, social and ethical issues associated with vitamin B12 testing was limited; therefore, the scope was broadened to include issues associated with vitamin tests and diagnostic tests in general. Many of these issues are likely applicable to vitamin B12 tests, albeit the potential impact is minimised owing to the relatively innocuous nature of the test.

No legal issues associated with vitamin B12 testing were identified.

Patients generally have a positive perception of vitamin B12 tests and vitamin testing more broadly. However, there was a disconnect between patients' expectations of testing and clinical recommendations by their GPs. This sometimes led to dissatisfaction with, and distrust of the GP.

Physicians acknowledge both medical and non-medical reasons for ordering vitamin B12 tests. The non-medical indications and ambiguous nature of vitamin B12 deficiency may contribute to the high rate of testing. Educational programs, updating clinical guidelines, improving patient–physician communication, and enabling longer consultations may aid in reducing unnecessary testing.

All tests have a degree of uncertainty, which contributes to false negative and false positives. The harm caused by these erroneous outcomes is minimised in the context of vitamin B12 because the condition is relatively easily addressed through supplementation, treatments and tests are safe, and physicians are likely to prescribe supplementation for borderline cases of deficiency.

7.2 Methods

Literature identified from systematic and non-systematic searches was used to address legal, social and ethical issues. Search terms used for the systematic search are outlined in ***Appendix A***, ***Table 34*** to ***Table 36***. The non-systematic search involved targeted searches of Google and PubMed using the following terms: 'access', 'attitudes', 'autonomy', 'benefits', 'burden', 'perception', 'B12', 'vitamin B12', 'vitamin B12 deficiency' and 'vitamin testing'. The non-systematic searches were conducted by a single reviewer, who identified 15 additional studies. A PRISMA chart was not provided, owing to the use of systematic and non-systematic searches. Results of the literature searches have been summarised using narrative synthesis.

7.3 Evidence table

No studies evaluated legal issues.

The assessment of ethical and social issues included 22 studies (***Table 21***) consisting of primary (k = 14) and secondary (k = 8) research. Primary research studies were performed mostly in Sweden (k = 3), the Netherlands (k = 2) or Israel (k = 2), with frequently studied populations being GPs and patients with vitamin B12 deficiency. Primary research predominantly consisted of surveying or interviewing patients and physicians to ascertain attitudes, barriers and facilitators to vitamin B12 testing and treatments.

Secondary research studies included database analyses (k = 3), published literature reviews (k = 4), and an economic analysis of vitamin supplementation (k = 1). The reviews summarised the contemporary management of vitamin B12 deficiency, the database analyses aimed to evaluate the prescribing practices of physicians, and the economic analysis evaluated the cost of switching from IM to oral supplementation.

It is unclear to what extent the identified studies represent the Swiss context, owing to the lack of Swiss-specific studies and the publication dates of included trials. Several studies were published between 2000 and 2010, coinciding with the introduction of MMA and holoTC tests and oral supplementation, so it is unclear to what extent these publications reflect current practice. It is also unclear if studies evaluating prescribing practices in Canada are applicable to Switzerland, given management guidelines and reimbursement practices likely differ between the countries.

There was limited literature addressing ethical issues associated with vitamin B12 testing and supplementation. Most studies considered the broader context of diagnostic testing. Therefore, in the absence of literature addressing vitamin B12 tests, studies discussing diagnostic testing were included. The applicability of these studies varies, as the potential harm and consequences of vitamin B12 testing is comparatively minor compared to other diagnostic tests (e.g. genetic tests); however, the broader implications remain relevant and caveats are noted throughout the following section.

Table 21 Characteristics of included studies for legal, social and ethical issues

Author, year; country	Population; sample size	Design; follow-up; setting	Interview/survey topics
Andres 2004[17] France	NA	Literature review	Vitamin B12 deficiency management
Cham 2018[163] Australia	GPs specialists and registrars n = 128	Survey NR Rural and urban practices	Factors contributing to whether GPs supplement for borderline vitamin B12 deficiency
Cramer 2017[164] USA	National representative sample n = 34,525	Survey 12 months (2012) NR	Prevalence of vegetarianism and veganism for health reasons

Author, year; country	Population; sample size	Design; follow-up; setting	Interview/survey topics
Dokuzlar 2017[165] Turkey	Geriatric patients with vitamin B12 deficiency n = 335	Observational, cross-sectional NR Outpatient clinics	Association between vitamin B12 levels and frailty
Graham 2007[166] Canada	GPs, geriatricians and haematologists n = 762	Survey NR NR	Attitudes towards vitamin B12 supplements
Hofstede 2019[167] the Netherlands	GPs n = 21 Patients n = 22	Survey NR General practices and primary care networks	Barriers and facilitators to reducing unnecessary vitamin D and B12 testing
Kool 2020[168] the Netherlands	GPs n = 182	Survey NR Medical centres	GP attitudes and knowledge of low value healthcare
Kwong 2005[169] Canada	Patients with vitamin B12 deficiency n = 86	Survey NR Academic hospitals and community health centres	Patient perspective on switching from injection to oral vitamin B12
Langan 2017[3] USA	NA	Literature review	Vitamin B12 deficiency management
Lökk 2001a[170] Sweden	GPs and geriatricians n = 1,098	Survey NR Medical centres	Attitudes and knowledge of vitamin B12 among physicians
Lökk 2001b[171] Sweden	GPs n = 485	Survey NR Medical centres	Attitudes and knowledge of vitamin B12 among physicians
Ma 2018[172] Canada	NA	Retrospective, database analysis 12 months Calgary Laboratory Services	Utilisation of laboratory tests in Canada
Metaxas 2017[173] Switzerland	Patients with vitamin B12 deficiency n = 37	RCT, survey 4 weeks General practices	Effectiveness and preference for IM or oral vitamin B12
Nilsson 2002[174] Sweden	GPs and geriatricians n = 1,006	Survey NR Medical centres	Attitudes and knowledge of vitamin B12 among physicians
Shaked 2019[175] Israel	Healthy, asymptomatic patients n = 15	Survey Five medical centres	Attitudes to vitamin testing among healthy patients
Silverstein 2019[176] Canada	Prescriptions for intramuscular B12 n = 405,469	Retrospective database analysis 2011 to 2015 IC/ES	Analysis of IM vitamin B12 prescriptions
Sukumar 2019[15] UK	NA	Literature review NR	Vitamin B12 deficiency management

Author, year; country	Population; sample size	Design; follow-up; setting	Interview/survey topics
Tal 2011[177] Israel	Elderly patients n = 1,570	Retrospective cross-sectional study 2007 Multicentre	Association between vitamin B12 levels and mortality in hospitalised older adults
Van Walraven 1999[178] Canada	Elderly patients with vitamin B12 deficiency n = 23,651	Retrospective database analysis 1996 to 1997 Ontario Health Insurance Plan database	Analysis of vitamin B12 prescriptions
Vidal-Alaball 2006[179] Wales	NR	Cost minimisation analysis NR	Costs associated with switching from IM to oral supplementation
Vinker 2007[180] Israel	Primary care physicians n = 162	Survey 2002 to 2003 Single primary medical centre	Association between physician characteristics and utilisation of laboratory tests
Wong 2015[157] Hong Kong	NA	Literature review NA	Vitamin B12 deficiency management

Abbreviations
B12 = vitamin B12, **GP** = general practitioner, **IC/ES** = Institute for Clinical Evaluative Sciences, **IM** = intramuscular, **n** = number of participants, **NA** = not applicable, **NR** = not reported, **RCT** = randomised controlled trial, **USA** = United States of America.

7.4 Legal results

No legal issues were identified from systematic and non-systematic searches.

7.5 Social results

7.5.1 Patient perception of vitamin tests and treatments

Healthy, asymptomatic patients generally have a positive perception of vitamin testing and use tests to validate their health and lifestyle and alleviate health-related anxiety.[175] Patients are often insistent on vitamin testing and will expect a referral for testing even if their physician explains why this is unnecessary. Thus, patients' perceptions may conflict with current medical recommendations suggesting patients should refrain from being tested if they are feeling well. It is unclear why patients' perceptions of vitamin testing have shifted; however, increasing media attention has been suggested to be a key contributing factor.[167 175]

Of patients referred for vitamin D or B12 testing (i.e. have presented with fatigue, depression, weight loss, myalgia or are part of a risk group) many have had negative experiences with their GP.[167] Patients believed that their GP had insufficient knowledge of vitamin testing, leading to distrust and dissatisfaction. Patients were also unhappy with physicians who refrained from testing and would continue to request testing until they received a referral. Many patients persisted because they believed there was no suitable alternative to vitamin testing, however, other surveyed patients were satisfied if

their GP was able to sufficiently explain why testing was not required. To improve satisfaction further, patients wanted GPs to provide more testing and pay more attention to vitamin deficiencies in practice.[167]

Patients indicated for vitamin testing predominately sourced their information from the internet and were willing to accept this information as it provided an explanation for their symptoms. However, they were confused by the differing thresholds for vitamin deficiency and the discordance between published guidelines. These concerns undermined their confidence in their GP because they found it difficult to accept that the GP had sourced the correct information.[167]

Patients with unspecified vitamin B12 deficiency were satisfied with oral supplementation and IM B12 injections with no clear preference between the two treatments.[169 173] When compared to IM injections, patients receiving oral supplementation reported reduced travel and healthcare visits as key drivers of satisfaction.[169] Oral supplement users further reported additional benefits of the avoidance of needles and complications associated with injections.[173] Patients who preferred IM injections believed that oral therapy reduced compliance, was more inconvenient and had questionable efficacy.[169 173]

Patients with pernicious anaemia were dissatisfied with their treatment for vitamin B12 deficiency (mostly IM injections). The reasons underscoring this dissatisfaction were not reported.[181]

7.5.2 Physician perception of vitamin tests and treatments

Vitamin B12 tests are among the most prescribed tests in clinical practice, with GPs ordering a median of two tests per week.[168] GPs acknowledge the high rate of testing and note that vitamin B12 tests cannot be performed for every case of suspected vitamin B12 deficiency.[168] They are also aware of the sensitivity and specificity limitations of vitamin B12 tests.[170 171] As such, GPs believed one diagnosis for every ten tests was an acceptable testing rate.[170]

Physicians are aware of the importance of reducing low-value healthcare, particularly vitamin B12 tests, to improve the quality and cost-efficiency of the healthcare system.[167 168] However, GPs note it is difficult to convince patients to refrain from testing. One survey reported only 9% of patients could be convinced to withhold from vitamin testing.[168] GPs note low education levels and limited language capabilities are key barriers underscoring the reluctance of patients to refrain from testing.[167] Additional barriers identified by GPs include inconsistent guidelines and recommendations, the lack of association between vitamin levels and symptoms, peer attitude towards testing, and the perceptions and hype towards vitamin testing in the media.[167 168] Important facilitators identified to reduce unnecessary testing were additional education programs, updated clinical guidelines, improved communication strategies, longer consultation times, removal of vitamin B12 tests from testing sets and feedback.[167]

Physicians acknowledge there are both medical and non-medical reasons for ordering vitamin B12 tests. Medical indications for testing vitamin B12 levels include low haemoglobin, neurological symptoms or the presence of certain risk factors (e.g. vegetarian or vegan diet).[167 170 171] Non-medical reasons for testing include providing reassurance to patients, acceding to patients' requests and insufficient time to discuss alternatives or justify why testing is unnecessary.[167] Other physician-related factors associated with an increased propensity to prescribe vitamin B12 tests include female GP, geriatricians, larger urban clinics and greater numbers of vitamin B12-deficient patients.[163 167 170 171 174 180]

There was relatively little information regarding the utilisation of vitamin B12 treatments by physicians. Despite the relatively high testing rates, it is unclear if prescriptions for vitamin B12 are equally high, with one survey noting most physicians had prescribed 10 or fewer oral vitamin B12 supplements within the previous year (noting, the number of IM B12 was not reported in the study).[166] Canadian physicians preferred IM treatments compared to oral supplements, with approximately half of surveyed clinicians exclusively using IM B12, although the indication for supplementation was not reported.[166]

Physicians generally held positive perceptions towards oral vitamin B12 supplementation.[166] Physicians who believed oral supplementation was effective thought it would reduce costs and avoid discomfort associated with injections. Additionally, physicians who believed oral supplementation was not efficacious raised concerns that patients would not be able or willing to pay for the medication; they noted single injections were more convenient and likely result in higher compliance than daily tablets and they were unsure about the existing literature.[166] Further, many physicians reported patients may not accept switching from injections to oral therapy and they were unsure which oral supplement to use. It is unclear to what extent these findings can be generalised to Swiss practice as oral supplementation appears to be more accepted (as inferred by its inclusion into the MediX guidelines[38]) since the publication of these surveys.

There was relatively little information regarding when to prescribe vitamin B12 in Switzerland. The lack of guidelines and accepted treatment thresholds compound this problem (see ***Section 10***). When considering patients with borderline vitamin B12 deficiency, Australian GPs were in favour of initiating vitamin B12 supplementation, providing additional dietary advice and subsequent retesting.[163] GPs would not recommend or were neutral toward testing additional biomarkers. GPs who did not perceive an overuse problem and those who were more receptive to patients' requests were more likely to prescribe vitamin B12 supplements. Factors such as the sex of the physician, elapsed time since last vitamin B12 education and the frequency of evaluating vitamin B12 deficiency, did not influence likelihood to prescribe vitamin B12.[163]

7.6 Ethical results

7.6.1 Vulnerable patient groups

Malnutrition and vitamin B12 disorders are relatively common among older adults, with prevalence increasing with age.[178 182] As with younger patients, symptoms of vitamin B12 deficiency in older patients are relatively non-specific and often undetectable, however, the cause differs slightly.[17 157] Dietary and lifestyle factors are common causes of deficiency in younger adults. In contrast, pernicious anaemia, GI disorders (gastritis and *Helicobacter pylori* infections) and previous GI surgery are common factors contributing to vitamin B12 deficiency in older adults.[17 157] Vitamin B12 deficiency in older adults may increase the risk of developing neuropsychiatric illnesses;[157] however, it is not associated with frailty[165] or mortality,[177] suggesting the impact of vitamin B12 deficiency is limited.

Individuals who are vegetarian or vegan are at greater risk of developing vitamin B12 deficiency because natural sources of vitamin B12 are generally limited to animal products.[183 184] The incidence of vitamin B12 deficiency in these populations ranges from 0% to 81%[185] and given the increasing popularity of these lifestyles,[164] overall incidence is likely to increase in the coming years.

Individuals who are pregnant or breastfeeding, neonates and children are also at risk of vitamin B12 deficiency.[184] Vitamin B12 requirements increase during pregnancy in order to meet the nutritional demands of the mother and the foetus. Deficiency is particularly harmful during this period as vitamin B12 is necessary for the myelination of neurons and neurodevelopment, and deficiency can result in delayed growth.[15] Given that the presentation of vitamin B12 deficiency is relatively non-specific in children and they are dependent on their caregiver for food, children and neonates represent a particularly vulnerable group.

7.6.2 Perceived benefits and harms of vitamin B12 testing

Non-maleficence and beneficence of diagnostic tests are linked to the method of testing and the specificity and sensitivity of the test. All tests have a degree of uncertainty (see ***Section 6.7*** for sensitivity and specificity of vitamin B12 tests), which will influence the rate of false positives and false negatives. Both misclassifications may harm the individual, with less sensitive tests likely to cause greater harm.

False positives can result in further tests or treatments in otherwise healthy patients and thus expose the individual to additional harm.[186] In vitamin B12 deficiency, the severity of false positives is relatively minor, as the harms and costs attributable to further testing (blood tests) and treatments (supplementation) are minimal. False positives may also confer psychological harm due to anxiety and stigma associated with the disorder, and cause patients to lose confidence in their medical practitioner.[186] Again, these harms are minimised because vitamin deficiencies are readily correctable using supplements. While the potential for harm is low, costs associated with an incorrect diagnosis

remain, specifically, the costs associated with supplementation. This will disproportionately impact individuals of low socioeconomic status and may result in psychological harm.

False negatives may result in treatments being delayed or withheld due to an incorrect diagnosis. This may result in the individual developing adverse events associated with vitamin B12 deficiency. These adverse events are often irreversible (owing to neuronal death) and would result in ongoing disability and costs[15] (see ***Section 6.7.3*** for further details). The costs are likely exacerbated in infants and young children who have a longer life expectancy. If the correct diagnosis is subsequently ascertained, treatment efficacy may be altered, additional treatments may be required and the prognosis may be poorer.[186] However, the harm associated with false negatives are potentially minimised for vitamin B12 deficiency given it takes several years to deplete B12 reserves, multiple testing may correct the diagnosis, physicians are likely to prescribe supplementation for borderline cases of deficiency, and deficiency can be readily addressed through supplementation.[15 163]

Irrespective of the result, the testing process itself can cause physical harm due to the test procedure or mental harm owing to the anxiety and stigma associated with the diagnosis. In the case of vitamin B12 deficiency, potential adverse effects of the test are outweighed by the potential benefit to the individual with suspected deficiency, with early diagnosis and treatment mitigating any possible complications attributable to the disorder. Treatment for vitamin B12 deficiency is relatively safe and affordable. However, patients not indicated for the test, are exposing themselves to additional harm—albeit relatively minor—which is unlikely to improve their overall health (noting it may alleviate anxiety).

8 Organisational issues

8.1 Summary statement organisational issues

If vitamin B12 testing were to be delisted or limited, physicians would likely initiate supplementation in patients at risk of, or suspected of, vitamin B12 deficiency without prior testing. This is unlikely to cause additional harm, but may increase unnecessary supplementation use.

Studies have reported patients have a willingness to transition from injections to oral supplementation; however, rates of compliance may decrease. Currently, there are no registered oral vitamin B12 products reimbursed in Switzerland.

Utilisation of vitamin B12 tests and IM injections has increased over the past several years in both Switzerland and Canada. Many of the tests and treatments are considered unnecessary and may reflect the increased medicalisation of vitamins.

8.2 Methods

Literature identified from systematic and non-systematic searches was used to address organisational issues. The search terms used for the systematic search are outlined in ***Appendix A***, ***Table 37***. The non-systematic search involved targeted searches of Google and PubMed using the following terms: ‘medicalisation’, ‘prevalence’, ‘prescribing’, ‘utilisation’, ‘B12’, ‘vitamin B12’, ‘vitamin B12 deficiency’ and ‘vitamin testing’. The non-systematic searches were conducted by a single reviewer, who identified four additional studies. A PRISMA chart was not provided owing to the use of systematic and non-systematic searches. Results of the literature searches have been summarised using narrative synthesis.

8.3 Evidence table

The assessment of organisational issues included primary (k = 4) and secondary research studies (k = 4) (***Table 22***). The primary research studies surveyed physicians or patients to identify barriers and facilitators to vitamin testing and gauge their willingness to transition from IM B12 injections to oral supplements. Secondary research consisted of a SR of the safety and effectiveness of vitamin B12 supplementation and retrospective analyses of databases to determine the prescribing and utilisation of vitamin B12 tests and injections in Switzerland and Canada.

Table 22 Characteristics of included studies for organisational issues

Author, year; country	Population; sample size	Design; follow-up; setting	Interview/survey topics
Cham 2018[163] Australia	GP, specialists and registrars n = 128	Survey NR Rural and urban practices	Factors contributing to whether GPs supplement for borderline vitamin B12 deficiency
Hofstede 2019[167] the Netherlands	GPs n = 21 Patients n = 22	Survey NR General practices and primary care networks	Barriers and facilitators to reducing unnecessary vitamin D and B12 testing
Huber 2020[154] Switzerland	Patients with a healthcare claim n = 997,761	Retrospective, database analysis 2012 to 2018 Helsana Group database	Utilisation of laboratory tests in Switzerland
Kwong 2005[169] Canada	Patients with vitamin B12 deficiency n = 86	Survey NR Academic hospitals and community health centres	Patient perspective on switching from injection to oral vitamin B12
Lökk 2001a[171] Sweden	GPs and geriatricians n = 1,098	Survey NR Medical centres	Attitudes and knowledge of vitamin B12 among physicians
Silverstein 2019[176] Canada	Prescriptions for IM B12 n = 405,469	Retrospective database analysis 2011 to 2015 IC/ES	Analysis of IM vitamin B12 prescriptions
Vidal-Alaball 2006[179] Wales	NR	Cost minimisation analysis NR	Costs associated with switching from IM to oral supplementation
Wang (2018)[8] China	Patients indicated for vitamin B12 supplementation n = 153	Systematic review NA	Safety and effectiveness of vitamin B12 supplementation

Abbreviations
B12 = vitamin B12, **GPs** = general practitioners, **IC/ES** = Institute for Clinical Evaluative Sciences, **IM** = intramuscular, **n** = number of patients, **NA** = not applicable, **NR** = not recorded.

8.4 Organisational results

8.4.1 Impact on healthcare resources

Limiting or restricting access to vitamin B12 tests would likely shift resource usage from tests to supplements. For example, following an online program to reduce low-value healthcare services in the Netherlands, GPs were more likely to suggest supplementation rather than testing.[167] Further, when considering a borderline case of vitamin B12 deficiency, Australian GPs were more likely to initiate supplementation and were ambivalent to, or would not recommend, further testing.[163] Lastly, a survey of Swedish GPs showed that they would prophylactically treat patients at risk of vitamin B12 deficiency,[171] reinforcing the notion that physicians would likely initiate supplementation if testing were

restricted. An increased rate of supplementation is unlikely to cause harm, owing to the relatively safe profile of vitamin B12.[8] Increased supplementation may result in many patients using vitamin supplements unnecessarily.

Delisting or limiting vitamin B12 tests may also switch the first-line treatment from injections to oral supplements. When surveyed, approximately 75% of patients indicated a willingness to transition from IM to oral supplementation.[169] Patients reported reduced travel time, costs, and healthcare visits as key reasons for their satisfaction with switching from IM to oral supplementation.[169] Other studies noted a transition from IM to oral supplementation would result in further healthcare savings due to reduced nursing time.[179] Importantly, oral and IM vitamin B12 exhibit equivalent safety and effectiveness.[8] Compliance is lower with oral therapy, however, with a survey of Canadian patients noting 92% had forgotten to take the medication in a six-month period.[169] This may result in inadequate treatment and additional healthcare costs from ongoing management of the disorder.

8.4.2 Medicalisation of vitamin supplementation and testing

Public interest in vitamins and vitamin testing has increased substantially over the past ten years as inferred by google trend searches for 'vitamin B12'.[187] Correspondingly, there has been increased demand and prescribing of vitamin B12 tests in Switzerland.[154] It is unclear if the incidence of vitamin B12 deficiency has also increased; many tests may have been unnecessary, merely reflecting public demand. In support of this possibility, approximately 75% of surveyed GPs had ordered one or more unnecessary vitamin B12 test in the previous week.[167] In Canada, 64% of vitamin B12 injections were considered inappropriate based on the patient's symptoms and prior vitamin B12 levels.[176]

While the medicalisation of vitamins may improve an individual's health status, unnecessary testing and treatments can further increase healthcare expenditure. For example, in Canada, the estimated cost associated with unnecessary vitamin B12 injections was 45.6 million in 2018.[176] There are several reasons underscoring this increased medicalisation, for example, increased media attention, patient awareness and willingness of physicians to maintain the patient-doctor relationship likely contribute to the over-testing and medicalisation of vitamins.[167]

9 Additional issues

Clinical practice guidelines (published after 2009) were sought via non-systematic search for inclusion in this report. Guidelines published in countries similar to Switzerland are a good source of information on the management of vitamin B12 deficiency in similar populations, and may highlight notable issues similar among health care systems. Five guidelines and one expert consensus statement were identified from the literature (***Table 23***).[3 16 31 38 188 189] Additional treatment management pathways (predominantly from the National Health Service) and expert opinions were also identified; however, these were omitted because they failed to provide recommendations or did not provide sufficient explanation or evidence justifying their pathway.

The guidelines were from Europe (k = 3), North America (k = 2), and Australia (k = 1). One guideline was from Switzerland, however, it is unclear how closely this is followed in practice. Most of the guidelines and the consensus statement were reflective of adults with a clinical suspicion of, or at high risk for, vitamin B12 deficiency. One guideline evaluated suspected vitamin B12 deficiency in pregnant women.

Most guidelines were in accordance with their recommendations for first-line tests, namely serum vitamin B12 tests and/or blood cell count/blood film examination. However, there is a lack of consensus regarding second-line tests and thresholds to infer vitamin B12 deficiency. For example, several guidelines recommend plasma or serum MMA, Hcy or holoTC tests, whereas other guidelines do not mention the tests or suggest holoTC as a first-line test. It is unclear why the guidelines differ in their recommendations and it is uncertain whether it is reflective of regional differences in clinical practice. Furthermore, the diagnostic threshold to infer deficiency using serum vitamin B12 levels varies between <75 pmol/L to <178 pmol/L. Likewise, the threshold for deficiency using MMA varied in two guidelines. The reason underscoring the different threshold is uncertain, but it may relate to the different testing kits (which influences the diagnostic accuracy) or the baseline vitamin B12 in the studied population.

Table 23 Summary of clinical guidelines and expert consensus regarding vitamin B12 testing

Author year; country; publisher	Population recommended for testing	Strength of recommendation	Recommended test/s (order of tests)	Strength of recommendation	Thresholds indicating vitamin B12 deficiency	Treatments	Strength of recommendation
Burkhart & Huber 2019[38] Switzerland MediX (network of medical clinics; non-peer reviewed guideline)	Patients with prior gastric resection, neurological symptoms (dementia, paraesthesia, polyneuropathy), glossitis have clinically suspected macrocytosis, or show signs of malnutrition. Benefit for general screening has not been established.	Recommended	1. Serum vitamin B12 and folate ** 1. HoloTC ** 2. MMA * 3. Anti-intrinsic factor antibodies	Recommended	*1. Serum vitamin B12* Deficiency: <150 pmol/L Possible deficiency (grey area): 150 to 250 pmol/L with high clinical suspicion of deficiency *HoloTC* No threshold defined *2. MMA* Deficiency: >0.27 µmol/L	Initiate treatment if confirmed deficiency or possible deficiency. Intramuscular or subcutaneous injection 1,000 µg per day 1, 3 and 5 or day 1 to 5, then weekly for 1 month, every 1 to 3 months thereafter. Oral vitamin B12 (1,000 µg) can also be used.	NR
Devalia 2014[16] UK British Haematological Society	Patients with the following risk factors: infections (relating to H. *Pylori*, G. *lamblia*, fish tapeworm), pernicious anaemia, gastric resection, coeliac disease, tropical sprue, Crohn disease, inadequate dietary intake, genetic disorders, and are	NR	1. Blood film examination 1. Serum vitamin B12 and folate 2. HoloTC [b] 2. Plasma tHcy and MMA* 3. Anti-intrinsic factor	Grade 2B Grade 1A Grade 1B Grade 2B	*1.Serum vitamin B12* Probable deficiency: <148 pmol/L [a] Possible vitamin B12 deficiency: >148 pmol/L with clinical suspicion of deficiency (risk factors)	Treatment as per British National Formulary recommendations [c] Intramuscular injection 1,000 µg, 3 times per week for 2 weeks, every 3 months thereafter. If neurological symptoms present, 1,000 µg on alternate days until no further improvement.	Grade 1A

Author year; country; publisher	Population recommended for testing	Strength of recommendation	Recommended test/s (order of tests)	Strength of recommendation	Thresholds indicating vitamin B12 deficiency	Treatments	Strength of recommendation
	pregnant or lactating.		antibody tests	Grade 2A	*2. MMA, tHcy and holoTC* (no consensus on thresholds)	Oral supplementation is not appropriate in pernicious anaemia but may be appropriate in maintenance or correction of suboptimal levels.	Grade 2C
Langan 2017[3] USA American Family Physician	Do not screen patients at average risk for deficiency. Screen patients with the following risk factors: vegan or vegetarian, alcohol abuse, gastric surgery, IBD, megaloblastic anaemia, metformin use >4 months, PPI use >12 months and histamine H_2 blocker use for >12 months.	NR C	1. Blood cell count and serum vitamin B12 2. MMA * 3. Anti-intrinsic factor antibodies 4. Serum gastrin	C C NR NR	*1.Serum vitamin B12* Deficiency: <111 pmol/L Low-normal: 111 to 294 pmol/L with clinical suspicion of deficiency (risk factors) Normal: ≥296 pmol/L 2. *MMA* No threshold defined	Intramuscular or oral supplementation are effective supplementation methods. If neurological symptoms are present or the patient has severe deficiency, use intramuscular injections. Patients who have had bariatric surgery should receive 1,000 µg of oral vitamin B12 per day indefinitely	B C
WA Government 2015[189] Australia King Edward	Individuals who are pregnant and are at increased risk of deficiency [d], have unexplained anaemia or fail to respond to treatment for iron deficiency anaemia	NA	1. Serum vitamin B12 2. HoloTC [e]	NA	*1.Serum vitamin B12* Deficiency: <110 pmol/L 2. HoloTC Threshold not defined	If there is a strong suspicion of deficiency, initiate oral supplementation with further investigation by GP. For vitamin B12 deficiency, 1 intramuscular injection 1,000 µg per week for 3 weeks.	NA

Author year; country; publisher	Population recommended for testing	Strength of recommendation	Recommended test/s (order of tests)	Strength of recommendation	Thresholds indicating vitamin B12 deficiency	Treatments	Strength of recommendation
Memorial Hospital						More frequent if severely deficiency or neurological symptoms present.	
Guidelines and Protocols Advisory Committee 2013[188] Canada British Columbia Guidelines	Test patients with unexplained neurological symptoms or macrocytic anaemia. Consider testing patients with gastrointestinal disorders or prior gastrointestinal surgery, use H_2 receptor antagonists (>12 months), PPI (>12 months) or metformin (>4 months), who are vegan or are older adults (>65 years).	NA	1. Blood film 1. Serum B12 test	NA	*1.Serum vitamin B12* High risk of deficiency: <75 pmol/L moderate risk of deficiency: 75 to 150 pmol/L Low risk of deficiency: 150 to 200 pmol/L Rare risk of deficiency: >220 pmol/L	Oral vitamin B12 (250 µg to 1,000 µg) is preferred treatment. If neurological symptoms are present, intramuscular or subcutaneous injection 1,000 µg, 1 to 5 injections per day followed by oral supplementation 1,000 µg to 2,000 µg daily. Prophylactic supplementation is recommended for strict vegans, food-bound vitamin B12 malabsorption and pernicious anaemia.	NA
European Food Safety Authority 2015[31] Europe Expert consensus	NR	NA	Serum vitamin B12 HoloTC MMA Plasma tHcy	Supportive biomarker Most specific marker Supportive biomarker Supportive biomarker	*Serum vitamin B12* lower limits: 134 to 178 pmol/L (adults) 108 to 242 pmol/L (children) *holoTC* lower limits: 11 to 48 pmol/L (adults) 26 to 38 pmol/L	NR	NA

Author year; country; publisher	Population recommended for testing	Strength of recommendation	Recommended test/s (order of tests)	Strength of recommendation	Thresholds indicating vitamin B12 deficiency	Treatments	Strength of recommendation
					(children) *MMA* upper limits: >0.21 to 0.41 µmol/L (adults) ≥0.3 µmol/L (children) *Plasma tH*cy no consensus (potentially 15 µmol/L)		

Abbreviations

GP = general practitioner, **holoTC** = holotranscobalamin II, **IBD** = inflammatory bowel disease, **MMA** = methylmalonic acid, **NA** = not applicable, **NR** = not reported, **pmol/L** = picomoles per litre, **PPI** = proton pump inhibitor, **tHcy** = total homocysteine, **µg** = micrograms, **µmol/L** = micromoles per litre.

Notes

Evidence rating for Langan (2017),[3] A = consistent, good-quality patient-oriented evidence, B = inconsistent or limited-quality patient-oriented evidence; C = consensus, disease oriented evidence, usual practice, expert opinion, or case series.

Evidence rating for Devalia (2014)[16] Grade 1: strong recommendations are made when there is confidence that the benefits do or do not outweigh harm and burden. Grade 2: where the magnitude of benefit or not is less certain. Quality of evidence – A: high, further research is very unlikely to change confidence in the estimate of effect (derived from RCTs without limitations). B: moderate, further research may have an important impact on confidence in the estimate effect (derived from RCTs with limitations or strong observation studies and case series). C: low, further research is likely to have an important impact on confidence in the estimate of effect and is likely to change the estimate.

***** when serum vitamin B12 or holoTC level is normal or low-normal.

****** only useful if there is clinical or laboratory suspicion of vitamin B12 deficiency.

a = or one derived from a local reference range, **b** = has the potential as a first-line test in the future, but an indeterminate 'grey area' exists, **c** = additional recommendations available for specific risk groups, for further information refer to Devalia 2014,[16] **d** = Patients who are older, are on a vegetarian or vegan diet, on medications (H2 receptor antagonists and biguanides) or have had prior gastrointestinal surgery, history of coeliac disease or IBD, **e** = test offered if patients present with equivocal or low total vitamin B12 levels (below 250 pmol/L in adults).

10 Discussion

This HTA short report aimed to evaluate the evidence for the effectiveness and safety of vitamin B12 testing. The tests under investigation included vitamin B12, holoTC or MMA tests of patient serum, plasma or urine. The review focused on initial testing to diagnose a vitamin B12 deficiency, rather than follow-up or monitoring tests. The safety and effectiveness of vitamin B12 supplementation was compared to no supplementation. Clinical management decisions regarding the route and frequency of the therapy were considered out of the scope of this report.

Across the evidence base, the serum vitamin B12 test was accepted as imperfect and prone to confounding factors. Additionally, inconsistencies within the literature were well recognised. The holoTC test has been proposed as a possible replacement as a first-line test to improve diagnostic accuracy in women >50 years.[33] Regardless of the first-line test, the total vitamin B12 or holoTC test often need to be supported with second-line testing in order to obtain highly sensitive and specific test results. ROC analyses reported highly variable sensitivities and specificities within the literature.[34] In summary, the sensitivity and specificity of each of the test vary widely and there is no general agreement on the type of reference standards to be used. Published summary estimated of sensitivity and specificity for serum vitamin B12 tests are hardly interpretable, in part due to the wide range of thresholds used and high concerns regarding applicability. It may be reasonable to use a sequential testing strategy involving several tests to reduce misclassifications, but such strategy has not yet been formally evaluated.

Studies evaluating how vitamin B12 tests affect management decisions in practice compared to current recommendations were examined in this report. Two primary studies suggested that the decisions made by health practitioners were not significantly influenced by the measured vitamin B12 level.[58] [59] Often the doctor did not follow official recommendations and proceeded to supplement (or not) irrespective of the test result. In contrast, a single study based across 10 GPs found that vitamin B12 tests were considered and significantly influenced clinical management decisions.[60]

There is ongoing debate in the literature regarding the route and frequency of supplementation, however this is not a focus of the report. Vitamin B12 supplementation was demonstrated as an effective treatment of a vitamin B12 deficiency in populations with vitamin B12 deficiency resulting from GI disease, pernicious anaemia, or of unspecified cause. The natural course of the disease can lead to an array of conditions ranging from mild to severe and permanent. A considerable portion of the evidence base was focused on the progression of deficiency in pregnant women and in infants.

10.1 Comparison to prior reviews and HTA reports

The Australian Government Department of Health (2014)[190] produced an HTA for the assessment of vitamin B12 testing. This HTA relied primarily on the Willis (2011)[34] review described in the current report. In summarising the evidence, the HTA noted that the diagnosis of conditions potentially amenable to vitamin B12 supplementation on the basis of a single serum vitamin B12 test is not an accurate way to identify those who are deficient. In terms of the performance of the holoTC test, the Australian Government Department of Health (2014) drew on nine primary studies that compared the performance of holoTC to that of total vitamin B12 for identification of patients with vitamin B12 deficiency.[190] All studies but one reported that the holoTC test's performance was comparable to, or better than, that of the vitamin B12 test, although a number of limitations were identified. The conclusion was that the evidence was insufficient to establish holoTC testing as an alternative to either total serum vitamin B12, or levels of MMA or Hcy in the diagnosis of vitamin B12 deficiency.

Hvas (2003),[191] the Danish group that contributed to change in management studies to this report,[59 60] subsequently published an HTA. The report was based on their prior publications reporting on the clinical utility of MMA in Denmark. They concluded that Vitamin B12 and MMA testing rates increased despite a lack of evidence regarding their clinical benefits. Hvas (2002 and 2000)[59 60] describes MMA testing as technically accurate but limited, as age and renal insufficiency are strong confounding factors. The test has a high sensitivity but uncertain specificity. The report emphasised that MMA was not effective as a first-line test, however this was based on short-term studies.

Findings from the Australian and Danish HTAs support the conclusions drawn in this current report.

10.2 Limitations in the included SRs

10.2.1 Diagnostic accuracy

Willis (2011)[34] was judged as having a low risk of bias. The review did not discuss the likelihood of publication bias ***Table 46***. The review was limited by the poor quality of the included studies (as assessed by the QUADAS tool), by restricted inclusion of English published literature, and by the statistical model used to obtain summary estimates for sensitivity and specificity while insufficiently accounting for threshold differences.

10.2.2 Change in management

An HTA by Hvas (2003)[191] was judged to have a high risk of bias against the AMSTAR criteria (28%). The study performed a comprehensive literature search, and a list of included studies along with the study characteristics were provided, but was limited in all other domains. This demonstrated that primary studies were required to further support the findings ***Table 46***.

10.2.3 Effectiveness of change in management studies

Four of the 13 SRs satisfied more that 70% of the AMSTAR criteria.[8 119 122 125] Four of the 13 SRs performed poorly against the AMSTAR checklist, and satisfied less than 30% of criteria.[19 32 120 124]

In the SRs that assessed the natural course of vitamin B12 deficiency, the threshold to diagnose a vitamin B12 deficiency differed, with two of the studies defining deficiency to be a serum vitamin B12 level <150 pmol/L,[32 126] whereas the other three studies did not provide this information. A common confounding factor across the three SRs that focused on maternal vitamin B12 status,[124-126] was that maternal folate status was not adjusted for in the included studies, which could confound the results.

In the SRs that assessed the supplementation of vitamin B12, there was substantial heterogeneity, as methods of administration, dosage, and follow-up duration differed. Of note, the cut-off to define vitamin B12 deficiency differed across each SR. Three of the included SRs included very few studies,[8 119 121] making the results less generalisable to the populations of interest that were investigated. Additionally, a common limitation reported in multiple SRs was the small sample sizes of the included studies and lack of standardised reporting of outcome measures.[19 119 122]

10.3 Limitations in the primary studies

Across all domains of the linked analysis, the patient populations included in the studies could not be easily separated according to the two defined groups outlined in this review, namely, patients being tested based on a clinical suspicion of B12 deficiency, and those at a high risk of B12 deficiency. The patient populations in the included studies were largely from samples of patients tested without a defined reason for testing.

10.3.1 Diagnostic accuracy

Comparison of sensitivity and specificity of B12, holoTC, and MMA tests among studies is limited due to variable thresholds and reference standards used. There is a trade-off relationship between sensitivity and specificity at different thresholds. Consequently, thresholds for both the index test and the reference standard should be the same or very similar among studies to allow for the interpretation of summary point estimates of sensitivity and specificity. However, this was not the case among the identified evidence base, leading to a degree of uncertainty.

Two diagnostic accuracy studies used reference tests with unestablished internal validity (red cell cobalamin test and 4cB12 model). Furthermore, the 4cB12 model incorporates all four tests for vitamin B12 and its biomarkers (B12, holoTC, MMA, and Hcy). Thus, there is overlap between the reference test and index test. While Campos (2020)[33] included a large sample size, the lack of clinical data about the population increased the risk of bias. The inclusion criteria required all four tests to have been performed simultaneously on the samples. This potentially selects for participants with a particular presentation of

disease which lead to a deviation from the recommended testing cascade. The study design allowed for the direct comparison of index tests and allowed the effects of age as a confounding factor to be mitigated.

Diagnostic accuracy studies generally did not exclude samples/participants based on creatinine value or kidney function (excluding two of six studies).[113 116] However, abnormal renal function was often noted as a confounding factor,[33] and in three studies the effects of kidney function on the diagnostic accuracy was investigated.[114 115 117]

Clinical data was not always available or reported among the six studies. Pernicious anaemia anti-IF antibodies can interact with the assays, affecting sensitivity and specificity of the tests. For this reason, the proportion of patients included with anti-IF antibodies may be significant. Similarly, in no case was haptocorrin deficiency investigated, likely due to the lack of clinical data available. However, it was occasionally raised as a confounding factor.[33 117] Clinical data was limited for most studies. This was a limitation as testing thresholds were variable among patient groups.

Most studies recruited a large sample size; however, Schwarz (2015)[116] included a small sample size (n = 77). The smaller sample size for this study limited the impact of the findings. Two studies performed their analyses retrospectively.[33 115] Just one of six studies recruited patients from multiple centres.[113]

10.3.2 Change in management

The results of this component of the linked evidence were limited due to the small volume of studies focusing on change in management that were identified, and the age of the studies. Although the studies were generally of good quality, these limitations common among the studies limit the generalisability of the findings.

10.3.3 Natural course of disease

The primary studies investigating the natural course of vitamin B12 generally did not report whether they were prospectively or retrospectively performed, or whether consecutive patients were recruited into the trial. Additionally, very little information was provided to decipher whether the included participants were at a similar point in the disease being investigated, though all used a predefined criterion to diagnose vitamin B12 deficiency. Depending on the nature of the study, some studies reported their results narratively, and other reporting resulting using basic statistical comparison. Lastly, the lack of power calculations (in most trials) and the absence of appropriate statistical tests raised uncertainties as to how generalizable the results are to the broader intended population.

10.4 Methodological limitations

The number of patient subgroups who undergo vitamin B12 testing is extensive and often non-specific. Consequently, they were not able to be separated and, for the purpose of this review, have been pooled

as a broad population of individuals who require vitamin B12 testing due to clinical suspicion or being at risk of a vitamin B12 deficiency.

Due to the outcomes reported in the included studies, it was not possible to update the meta-analysis performed by Willis (2011).[34] No meta-analysis could be performed on the six primary accuracy studies published after the inclusion period of the Willis review (November 2009). Consequently, the conclusions of the evidence base drawn from narratively described outcomes may be limited. The Willis (2011) review focuses on serum B12 tests only.[34] Therefore, holoTC or MMA publications prior to 2009 may not have been captured; however, a non-systematic search for studies published prior to 2009 was performed to evaluate the risk of missing studies, and no studies fulfilling the inclusion criteria were identified.

The evidence-base for diagnostic accuracy and change in management studies does not specify whether testing of the patient was an initial test or prompted by monitoring of at-risk patients. Additionally, study characteristics were scarcely described. The inclusion criteria were limited to studies published in English, German, French or Italian with an English abstract. Consequently, studies published in other languages may have been missed in the screening process.

10.5 Legal, social, ethical and organisational issues

The social and organisational issues were related to the medicalisation of vitamins. Over the past 10 years the public's awareness of vitamin supplementation has increased.[187] The increased awareness reflects the positive portrayal of vitamin supplements in the media[192] and the pervading view that supplementation is necessary for maintaining a healthy lifestyle in the general population.[167 175] These factors have fostered a positive perception of vitamin B12 testing among patients and has likely contributed to the increased demand for vitamin testing. However, it is unclear whether the incidence of vitamin B12 deficiency has increased. Physicians acknowledged the increased demand and utilisation of vitamin B12 tests and admitted finding it difficult to convince patients to refrain from testing (which often results in negative experiences for patients).[167 168] This further increases the number of unnecessary tests. However, physicians also note that complying with a patient's request has some merit because providing non-indicated tests strengthens their relationship with the patient.[167] Strategies to reduce unnecessary testing in practice relate to improved educational resources and guidelines, longer consultation times, and removal of vitamin B12 tests from testing sets.[167]

If vitamin B12 tests were limited, physicians would likely initiate supplementation earlier in the treatment pathway. This would likely reduce healthcare expenditure but likely increase out-of-pocket costs for the patient because oral supplementation is only reimbursed in Switzerland as a b-complex cocktail or multivitamin.[38] There is no reimbursed mono-B12 oral supplement. Importantly, patients are satisfied with treatments for vitamin B12 (including oral and IM).[169 173] Patients who prefer oral supplements report reduced travel and healthcare visits and the avoidance of painful injections as key drivers of their

satisfaction. Whereas patients who prefer IM supplementation noted oral therapy reduced compliance, was more inconvenient and had questionable efficacy.[169 173]

The key ethical concern related to the uncertainty associated with diagnostic tests. The sensitivity and specificity of vitamin B12 tests are variable (see ***Section 6***), resulting in false positives and false negatives and thus erroneous diagnoses. Incorrect diagnoses potentially harm patients, as they may develop adverse events owing to an untreated illness or they may undergo unnecessary treatments and tests.[186] In the context of vitamin B12 deficiency, the potential for harm owing to a false positive result is relatively minor. For example, subsequent blood tests and treatments (supplements) are safe and relatively innocuous.[8] False negative results have the potential to cause harm if the vitamin deficiency remains untreated owing to the development of severe adverse events (e.g. polyneuropathy, peripheral nerve dysfunction and sensory deficits). However, given physicians are likely to prescribe supplements for borderline cases,[163] it takes several years for vitamin B12 levels to deplete, and vitamin deficiency is relatively easily addressed through supplementation, the potential for harm owing to false negatives is reduced.[15]

11 Conclusions

Vitamin B12 tests reported moderately low sensitivity and specificity. The diagnostic accuracy was variable among the evidence base due to the reference tests and patient populations included. In patients with subclinical deficiency, vitamin B12 specificity was relatively similar between the Willis (2011)[34] review and the Swiss study by Campos (2020).[33] Sensitivity was comparatively reduced in results by Willis (2011), particularly when MMA was used as a reference test. The reported susceptibility of the tests to confounding factors such as age and renal function was variable.[33 34]

The current evidence does not inform the most appropriate test, or combination of tests, to investigate a patient with suspected, or high risk of, B12 deficiency, nor the utility of such tests on impacting patient outcomes. Due to the variable accuracy and utility of the individual index tests in the testing cascade, and the absence of an accepted reference test, designing an ideal diagnostic accuracy study is challenging. Therefore, the most useful study design to address this research question would be a randomised controlled trial, in which patients are randomised to receive testing (all tests, following the recommended testing cascade) or no testing, with patients in both groups followed-up over time to measure the impacts of differing treatment strategies on patient-relevant outcomes. In the absence of such evidence, further research into the optimal reference standard is needed in order for the diagnostic validity of different diagnostic pathways to be rigorously evaluated.

Vitamin B12 and MMA tests did not seem to change patient management in hospitals, however, they were used in decision-making in GP clinics. These findings were limited as they were from a small evidence base and over two decades old. Furthermore, how accurately the Danish and USA healthcare systems reflect Swiss healthcare during the testing cascade remains unclear. No study addressing holoTC testing was identified.

In patients that are correctly identified as B12 deficient, B12 supplementation was found to be effective at achieving a clinical response. Cases that are incorrectly classified as healthy (i.e. false negatives) could face mild to severe consequences (e.g., neurological abnormalities, cognitive impairment, haematological abnormalities, complications during pregnancy). Cases that are correctly identified as true positives could avoid unnecessary supplementation. The main social and organisational issues were related to the medicalisation of vitamins and the subsequent rise in unnecessary testing. To reduce unnecessary testing, physicians propose improved education resources, longer consultation times and removal of vitamin B12 tests from grouped testing kits. The ethical concerns related to erroneous diagnoses owing to false positives and false negatives. The impact of these erroneous outcomes is minimised in the context of vitamin B12 deficiency because supplements and blood tests are relatively safe and it takes several years for vitamin B12 reserves to deplete.

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Appendix A: Source of literature (databases and websites)

Table 24 Database search results (up to 18 October 2020)

Database	Search results
Embase and Medline	1,328
The Cochrane Library	66
York CRD	37
INAHTA HTA Database	11
Total	**1,442**

Table 25 Database search results, primary study searches (up to 4 November 2020)

Database	Search results
Embase and Medline	6,339
The Cochrane Library	192
Total	**6,531**

Table 26 Search strategy for systematic reviews in Embase and Medline (18 October 2020)

#	Search terms	Results
1	exp *Vitamin B 12 Deficiency/ use ppez	8254
2	exp *B12 deficiency/ use oemezd	2199
3	Vitamin B 12/df use ppez	0
4	Cobamides/df use ppez	17
5	Hydroxocobalamin/df use ppez	1
6	((((vitamin* or hypovitaminosis or hypo-vitaminosis) adj (B12 or B-12 or "b(12)")) or AdCbl or AdoCbl or adenosylcobalamin or avitaminosis or Cbl or CN-Cbl or cobalamin? or coballamin? or cobamides or cyanocobalamin? or deoxyadenosinecobalamin? or eritron or hydroxocobalamin? or hydroxo-cobalamin? or MeCbl or methylcobalamin? or methyl-cobalamin?) adj2 (deficien* or inadequa* or insufficien*)).ti,kf,kw.	5748
7	((B12 or B-12 or "b(12)" or AdCbl or AdoCbl or adenosylcobalamin or avitaminosis or Cbl or CN-Cbl or cobalamin? or coballamin? or cobamides or cyanocobalamin? or deoxyadenosinecobalamin? or eritron or hydroxocobalamin? or hydroxo-cobalamin? or MeCbl or methylcobalamin? or methyl-cobalamin?) and (deficien* or inadequa* or insufficien*)).ti.	6183
8	((pernicious or Addison*) adj anemi*).ti,kf,kw.	3076
9	or/1-8	14630
10	(exp Vitamin B 12/ and (exp Diagnosis/ or Hematologic Tests/ or Mass Screening/ or di.fs. or (assay? or (blood adj2 level?) or concentration? or detect* or diagnos* or level? or screen* or serum? or test*).tw,kf,kw.)) use ppez	11811
11	((Cyanocobalamin/ or Cobamamide/ or Hydroxocobalamin/) and (exp Analysis/ or exp Diagnosis/ or Diagnostic Procedure/ or exp Blood Examination/ or exp Screening/ or Vitamin Blood Level/ or di.fs. or (assay? or (blood adj2 level?) or concentration? or detect* or diagnos* or level? or screen* or serum? or test*).tw,kf,kw.)) use oemezd	29105
12	exp Vitamin B 12 Deficiency/bl, di use ppez	3293
13	exp B12 Deficiency/di use oemezd	636
14	(((vitamin* or hypovitaminosis or hypo-vitaminosis) adj (B12 or B-12 or "b(12)")) or AdCbl or AdoCbl or adenosylcobalamin or avitaminosis or Cbl or CN-Cbl or cobalamin? or coballamin? or cobamides or cyanocobalamin? or deoxyadenosinecobalamin? or eritron or hydroxocobalamin? or hydroxo-cobalamin? or MeCbl or methylcobalamin? or methyl-cobalamin?).tw,kf,kw. and di.fs.	6274
15	((((vitamin* or hypovitaminosis or hypo-vitaminosis) adj (B12 or B-12 or "b(12)")) or AdCbl or AdoCbl or adenosylcobalamin or avitaminosis or Cbl or CN-Cbl or cobalamin? or coballamin? or cobamides or cyanocobalamin? or deoxyadenosinecobalamin? or eritron or hydroxocobalamin? or hydroxo-cobalamin? or MeCbl or methylcobalamin? or methyl-cobalamin?) adj2 (assay? or (blood adj2 level?) or concentration? or detect* or diagnos* or level? or screen* or serum? or test*)).tw,kf,kw.	13391
16	((B12 or B-12 or "b(12)" or AdCbl or AdoCbl or adenosylcobalamin or avitaminosis or Cbl or CN-Cbl or cobalamin? or coballamin? or cobamides or cyanocobalamin? or deoxyadenosinecobalamin? or eritron or hydroxocobalamin? or hydroxo-cobalamin? or MeCbl or methylcobalamin? or methyl-cobalamin?) and (assay? or (blood adj2 level?) or concentration? or detect* or diagnos* or level? or screen* or serum? or test*)).ti.	5848
17	(Methylmalonic Acid/ and (exp Diagnosis/ or Hematologic Tests/ or Mass Screening/ or di.fs. or (detect* or diagnos* or screen* or test*).tw,kf,kw.)) use ppez	957
18	(Methylmalonic Acid/ and (exp Analysis/ or exp Diagnosis/ or Diagnostic Procedure/ or exp Blood Examination/ or exp Screening/ or Vitamin Blood Level/ or di.fs. or (detect* or diagnos* or screen* or test*).tw,kf,kw.)) use oemezd	2397
19	(methylmalonic acid? or methyl malonic acid? or MMA or methylmalonate? or methyl-malonate?).tw,kf,kw. and di.fs.	1409
20	((methylmalonic acid? or methyl malonic acid? or MMA or methylmalonate? or methyl-malonate?) adj5 (detect* or diagnos* or screen* or test*)).tw,kf,kw.	1199

#	Search terms	Results
21	(*Transcobalamins/ and (exp Diagnosis/ or Hematologic Tests/ or Mass Screening/ or di.fs. or (detect* or diagnos* or screen* or test*).tw,kf,kw.)) use ppez	315
22	(*Transcobalamins/ and (exp Analysis/ or exp Diagnosis/ or Diagnostic Procedure/ or exp Blood Examination/ or exp Screening/ or Vitamin Blood Level/ or di.fs. or (detect* or diagnos* or screen* or test*).tw,kf,kw.)) use oemezd	211
23	(holoTC or holotranscobalamin or holo-transcobalamin or active B12 or active B-12 or "active b(12)").tw,kf,kw. and di.fs.	186
24	((holoTC or holotranscobalamin or holo-transcobalamin or active B12 or active B-12 or "active b(12)") adj5 (detect* or diagnos* or screen* or test*)).tw,kf,kw.	123
25	or/10-24	50598
26	exp Vitamin B 12 Deficiency/dh, dt, th use ppez	2032
27	exp B12 deficiency/dm, dt, th use oemezd	661
28	Vitamin B 12/dt, tu, th use ppez	3706
29	CyanocobalamiNAd, dt use oemezd	6489
30	Cobamides/tu use ppez	67
31	Cobamamide/ad, dt use oemezd	70
32	Hydroxocobalamin/tu use ppez	393
33	HydroxocobalamiNAd, dt use oemezd	969
34	((exp B12 deficiency/ or Cyanocobalamin/ or Cobamamide/ or Hydroxocobalamin/) and (Diet Supplementation/ or Vitamin Supplementation/)) use oemezd	5401
35	((((vitamin* or hypovitaminosis or hypo-vitaminosis) adj (B12 or B-12 or "b(12)")) or AdCbl or AdoCbl or adenosylcobalamin or avitaminosis or Cbl or CN-Cbl or cobalamin? or coballamin? or cobamides or cyanocobalamin? or deoxyadenosinecobalamin? or eritron or hydroxocobalamin? or hydroxo-cobalamin? or MeCbl or methylcobalamin? or methyl-cobalamin?) adj5 (administ* or dose? or inject* or oral or orally or supplement* or therapy or therapies or therapeutic* or treat*)).tw,kf,kw.	10831
36	((B12 or B-12 or "b(12)" or AdCbl or AdoCbl or adenosylcobalamin or avitaminosis or Cbl or CN-Cbl or cobalamin? or coballamin? or cobamides or cyanocobalamin? or deoxyadenosinecobalamin? or eritron or hydroxocobalamin? or hydroxo-cobalamin? or MeCbl or methylcobalamin? or methyl-cobalamin?) and (administ* or dose? or inject* or oral or orally or supplement* or therapy or therapies or therapeutic* or treat*)).ti.	4243
37	or/26-36	23628
38	Meta-Analysis.pt.	121037
39	Meta-Analysis/ or Systematic Review/ or Meta-Analysis as Topic/ or "Meta Analysis (Topic)"/ or "Systematic Review (Topic)"/ or exp Technology Assessment, Biomedical/ or Network Meta-Analysis/	657616
40	(meta-analy* or metaanaly* or met-analy* or metanaly*).tw,kf,kw.	431134
41	(systematic review* or systematic overview* or evidence-based review* or evidence-based overview* or (evidence adj3 (review* or overview*)) or meta-review* or meta-overview* or meta-synthes* or rapid review* or "review of reviews" or umbrella review?).tw,kf,kw.	508393
42	(technology assessment* or HTA or HTAs or technology overview* or technology appraisal*).tw,kf,kw.	23669
43	((quantitative adj3 (review* or overview* or synthes*)) or (research adj3 (integrati* or overview*))).tw,kf,kw.	24908
44	((integrative adj3 (research or review* or overview*)) or (collaborative adj3 (review* or overview*)) or (pool* adj3 analy*)).tw,kf,kw.	69482
45	(meta regression* or metaregression*).tw,kf,kw.	21276
46	(systematic review* or biomedical technology assessment* or bio-medical technology assessment*).mp,hw.	569437

#	Search terms	Results
47	(cochrane or (health adj2 technology assessment) or evidence report or systematic review?).jw.	55040
48	((indirect or indirect treatment or mixed-treatment or bayesian) adj3 comparison*).tw,kf,kw.	9169
49	(multi* adj3 treatment adj3 comparison*).tw,kf,kw.	596
50	(multi* adj2 paramet* adj2 evidence adj2 synthesis).tw,kf,kw.	35
51	(multiparamet* adj2 evidence adj2 synthesis).tw,kf,kw.	35
52	(multi-paramet* adj2 evidence adj2 synthesis).tw,kf,kw.	29
53	or/38-52	1015502
54	9 and 53	135
55	limit 54 to (english or french or german or italian)	133
56	remove duplicates from 55	106
57	25 and 53	1250
58	limit 57 to (english or french or german or italian)	1230
59	remove duplicates from 58	1043
60	37 and 53	933
61	limit 60 to (english or french or german or italian)	918
62	remove duplicates from 61	790
63	56 or 59 or 62	1328

Table 27 Search strategy for systematic reviews in the Cochrane Library (18 October 2020)

#	Search terms	Results
1	MeSH descriptor: [Vitamin B 12 Deficiency] explode all trees	152
2	MeSH descriptor: [Vitamin B 12] explode all trees	887
3	((vitamin* OR hypovitaminosis OR hypo-vitaminosis) NEXT (B12 OR B-12 OR "b(12)")):ti OR ((vitamin* OR hypovitaminosis OR hypo-vitaminosis) NEXT (B12 OR B-12 OR "b(12)")):ab OR ((vitamin* OR hypovitaminosis OR hypo-vitaminosis) NEXT (B12 OR B-12 OR "b(12)")):kw	2227
4	(B12 OR B-12 OR "b(12)" OR cobalamin? OR cobamides OR cyanocobalamin? OR eritron OR hydroxocobalamin? OR methylcobalamin?):ti OR (B12 OR B-12 OR "b(12)" OR cobalamin? OR cobamides OR cyanocobalamin? OR eritron OR hydroxocobalamin? OR methylcobalamin?):kw	1891
5	MeSH descriptor: [Methylmalonic Acid] this term only	53
6	MeSH descriptor: [Transcobalamins] this term only	17
7	((methylmalonic acid? OR methyl malonic acid? OR MMA) NEAR/5 (detect* OR diagnos* OR screen* OR test*)):ti,ab,kw OR ((holoTC OR holotranscobalamin OR holo-transcobalamin OR active B12 OR active B-12 OR active "b(12)") NEAR/5 (detect* OR diagnos* OR screen* OR test*)):ti,ab,kw	5830
8	#1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7	8384
9	#8 limit to systematic reviews	66

Table 28 Search strategy for systematic reviews and HTA reports in York CRD (DARE, HTA) (18 October 2020)

#	Search terms	Results
1	MeSH DESCRIPTOR Vitamin B 12 Deficiency IN DARE,HTA	14
2	MeSH DESCRIPTOR Vitamin B 12 IN DARE,HTA	27
3	MeSH DESCRIPTOR Methylmalonic Acid IN DARE,HTA	2
4	MeSH DESCRIPTOR Transcobalamins IN DARE,HTA	2
5	(B12 OR B-12 OR "b(12)" OR AdCbl OR AdoCbl OR adenosylcobalamin OR avitaminosis OR Cbl OR CN-Cbl OR cobalamin? OR coballamin? OR cobamides OR cyanocobalamin? OR deoxyadenosinecobalamin? OR eritron OR hydroxocobalamin? OR hydroxo-cobalamin? OR MeCbl OR methylcobalamin? OR methyl-cobalamin?):TI OR ((methylmalonic acid? OR methyl malonic acid? OR MMA) NEAR5 (detect* OR diagnos* OR screen* OR test*)):TI OR ((holoTC OR holotranscobalamin OR holo-transcobalamin OR active B12 OR active B-12 OR active "b(12)") NEAR5 (detect* OR diagnos* OR screen* OR test*)):TI IN DARE, HTA	27
6	#1 OR #2 OR #3 OR #4 OR #5	37

Table 29 Search strategy for systematic reviews and HTA reports in the INAHTA HTA Database (18 October 2020)

#	Search terms	Results
1	(("Vitamin B 12 Deficiency")[mhe] OR ("Vitamin B 12")[mhe] OR ("Methylmalonic Acid")[mh] OR ("Transcobalamins")[mh] OR (B12 OR B-12 OR "b(12)" OR adenosylcobalamin OR avitaminosis OR cobalamin* OR coballamin* OR cobamide* OR cyanocobalamin* OR deoxyadenosinecobalamin* OR eritron OR hydroxocobalamin* OR hydroxo-cobalamin* OR methylcobalamin* OR methyl-cobalamin* OR "methylmalonic acid" OR "methyl-malonic acid" OR holoTC OR holotranscobalamin)[Title])	11

Table 30 Search strategy for primary studies in Medline and Embase (4 November 2020)

#	Searches	Results
1	exp *Vitamin B 12 Deficiency/ use ppez	8266
2	exp *B12 deficiency/ use oemezd	2222
3	Vitamin B 12/df use ppez	0
4	Cobamides/df use ppez	17
5	Hydroxocobalamin/df use ppez	1
6	((((vitamin* or hypovitaminos#s or hypo-vitaminos#s) adj (B12 or B-12 or "b(12)")) or AdCbl or AdoCbl or adenosylcobalamin? or avitaminos#s or Cbl or CN-Cbl or cobalamin? or coballamin? or cobamides or cyanocobalamin? or deoxyadenosinecobalamin? or eritron or hydroxocobalamin? or hydroxo-cobalamin? or MeCbl or methylcobalamin? or methyl-cobalamin?) adj2 (deficien* or inadequa* or insufficien*)).ti,kf,kw.	5782
7	((B12 or B-12 or "b(12)" or AdCbl or AdoCbl or adenosylcobalamin? or avitaminos#s or Cbl or CN-Cbl or cobalamin? or coballamin? or cobamides or cyanocobalamin? or deoxyadenosinecobalamin? or eritron or hydroxocobalamin? or hydroxo-cobalamin? or MeCbl or methylcobalamin? or methyl-cobalamin?) and (deficien* or inadequa* or insufficien*)).ti.	6219
8	(((macrocytic hyperchromic or pernicious or primary or Addison*) adj2 an?emi*) or biermer disease).ti,kf,kw.	5763
9	or/1-8	15306

#	Searches	Results
10	(exp *Diagnosis/ or *Hematologic Tests/ or *Mass Screening/ or (assay? or (blood adj2 level?) or concentration? or detect* or diagnos* or level? or screen* or serolog* or serum? or test*).ti,kf,kw.) use ppez	4611864
11	(exp *Analysis/ or exp *Diagnosis/ or *Diagnostic Procedure/ or exp *Blood Examination/ or exp *Screening/ or *Vitamin Blood Level/ or (assay? or (blood adj2 level?) or concentration? or detect* or diagnos* or level? or screen* or serolog* or serum? or test*).ti,kf,kw.) use oemezd	5265660
12	or/10-11	9877524
13	9 and 12	3037
14	(exp Vitamin B 12/ and (exp Diagnosis/ or Hematologic Tests/ or Mass Screening/ or di.fs. or (assay? or (blood adj2 level?) or concentration? or detect* or diagnos* or level? or screen* or serolog* or serum? or test*).tw,kf,kw.)) use ppez	11838
15	((Cyanocobalamin/ or Cobamamide/ or Hydroxocobalamin/) and (exp Analysis/ or exp Diagnosis/ or Diagnostic Procedure/ or exp Blood Examination/ or exp Screening/ or Vitamin Blood Level/ or di.fs. or (assay? or (blood adj2 level?) or concentration? or detect* or diagnos* or level? or screen* or serolog* or serum? or test*).tw,kf,kw.)) use oemezd	29247
16	exp Vitamin B 12 Deficiency/bl, di use ppez	3298
17	exp B12 Deficiency/di use oemezd	639
18	(((vitamin* or hypovitaminos#s or hypo-vitaminos#s) adj (B12 or B-12 or "b(12)")) or AdCbl or AdoCbl or adenosylcobalamin? or avitaminos#s or Cbl or CN-Cbl or cobalamin? or coballamin? or cobamides or cyanocobalamin? or deoxyadenosinecobalamin? or eritron or hydroxocobalamin? or hydroxo-cobalamin? or MeCbl or methylcobalamin? or methyl-cobalamin?).tw,kf,kw. and di.fs.	6325
19	((((vitamin* or hypovitaminos#s or hypo-vitaminos#s) adj (B12 or B-12 or "b(12)")) or AdCbl or AdoCbl or adenosylcobalamin? or avitaminos#s or Cbl or CN-Cbl or cobalamin? or coballamin? or cobamides or cyanocobalamin? or deoxyadenosinecobalamin? or eritron or hydroxocobalamin? or hydroxo-cobalamin? or MeCbl or methylcobalamin? or methyl-cobalamin?) adj2 (assay? or (blood adj2 level?) or concentration? or detect* or diagnos* or level? or screen* or serolog* or serum? or test*)).tw,kf,kw.	13457
20	((B12 or B-12 or "b(12)" or AdCbl or AdoCbl or adenosylcobalamin? or avitaminos#s or Cbl or CN-Cbl or cobalamin? or coballamin? or cobamides or cyanocobalamin? or deoxyadenosinecobalamin? or eritron or hydroxocobalamin? or hydroxo-cobalamin? or MeCbl or methylcobalamin? or methyl-cobalamin?) and (assay? or (blood adj2 level?) or concentration? or detect* or diagnos* or level? or screen* or serolog* or serum? or test*)).ti.	5885
21	(Methylmalonic Acid/ and (exp Diagnosis/ or Hematologic Tests/ or Mass Screening/ or di.fs. or (assay? or detect* or diagnos* or screen* or test*).tw,kf,kw.)) use ppez	979
22	(Methylmalonic Acid/ and (exp Analysis/ or exp Diagnosis/ or Diagnostic Procedure/ or exp Blood Examination/ or exp Screening/ or Vitamin Blood Level/ or di.fs. or (assay? or detect* or diagnos* or screen* or test*).tw,kf,kw.)) use oemezd	2420
23	(methylmalonic acid? or methyl malonic acid? or MMA or methylmalonate? or methyl-malonate?).tw,kf,kw. and di.fs.	1418
24	((methylmalonic acid? or methyl malonic acid? or MMA or methylmalonate? or methyl-malonate?) adj5 (detect* or diagnos* or screen* or test*)).tw,kf,kw.	1207
25	(*Transcobalamins/ and (exp Diagnosis/ or Hematologic Tests/ or Mass Screening/ or di.fs. or (assay? or detect* or diagnos* or screen* or test*).tw,kf,kw.)) use ppez	338
26	(*Transcobalamin/ and (exp Analysis/ or exp Diagnosis/ or Diagnostic Procedure/ or exp Blood Examination/ or exp Screening/ or Vitamin Blood Level/ or di.fs. or (assay? or detect* or diagnos* or screen* or test*).tw,kf,kw.)) use oemezd	215
27	(holoTC or holotranscobalamin? or holo-transcobalamin? or active B12 or active B-12 or "active b(12)").tw,kf,kw. and di.fs.	191
28	((holoTC or holotranscobalamin? or holo-transcobalamin? or active B12 or active B-12 or "active b(12)") adj5 (assay? or detect* or diagnos* or screen* or test*)).tw,kf,kw.	163
29	or/14-28	50838

#	Searches	Results
30	9 and 29	7621
31	limit 9 to "prognosis (best balance of sensitivity and specificity)"	1456
32	exp Cohort Studies/ [both]	2687175
33	exp Disease Progression/ use ppez	183502
34	exp Disease Course/ use oemezd	3479332
35	exp Morbidity/ use ppez	566803
36	Morbidity/ use oemezd	350735
37	exp Mortality/ [both]	1493965
38	exp Outcome Assessment, Health Care/ use ppez	1151793
39	Prognosis/ [both]	1109088
40	exp Survival Analysis/ [both]	326355
41	exp Treatment Outcome/ use oemezd	1718499
42	course.ti.	155950
43	(cohort* or compar* or first episode? or followup or follow-up or longitudinal* or outcome* or predict* or prognos* or prospective* or survival).tw,kf,kw.	21189499
44	((natural* or disease*) adj (course* or deteriorat* or exacerbat* or evolution or history or progress*)).tw,kf,kw.	425818
45	or/32-44	24560043
46	9 and 45	3849
47	exp animals/	49949205
48	exp animal experimentation/ or exp animal experiment/	2641260
49	exp models animal/	2010512
50	nonhuman/	6391299
51	exp vertebrate/ or exp vertebrates/	48608059
52	or/47-51	51833721
53	exp humans/	40467641
54	exp human experimentation/ or exp human experiment/	537623
55	or/53-54	40470095
56	52 not 55	11365286
57	Meta-Analysis.pt.	121957
58	Meta-Analysis/ or Systematic Review/ or Meta-Analysis as Topic/ or "Meta Analysis (Topic)"/ or "Systematic Review (Topic)"/ or exp Technology Assessment, Biomedical/ or Network Meta-Analysis/	662392
59	(meta-analy* or metaanaly* or met-analy* or metanaly*).tw,kf,kw.	435010
60	(systematic review* or systematic overview* or evidence-based review* or evidence-based overview* or (evidence adj3 (review* or overview*)) or meta-review* or meta-overview* or meta-synthes* or rapid review* or "review of reviews" or umbrella review?).tw,kf,kw.	513483
61	(technology assessment* or HTA or HTAs or technology overview* or technology appraisal*).tw,kf,kw.	23765
62	((quantitative adj3 (review* or overview* or synthes*)) or (research adj3 (integrati* or overview*))).tw,kf,kw.	25080
63	((integrative adj3 (research or review* or overview*)) or (collaborative adj3 (review* or overview*)) or (pool* adj3 analy*)).tw,kf,kw.	70035
64	(meta regression* or metaregression*).tw,kf,kw.	21538

#	Searches	Results
65	(systematic review* or biomedical technology assessment* or bio-medical technology assessment*).mp,hw.	574996
66	(cochrane or (health adj2 technology assessment) or evidence report or systematic review?).jw.	55064
67	((indirect or indirect treatment or mixed-treatment or bayesian) adj3 comparison*).tw,kf,kw.	9222
68	(multi* adj3 treatment adj3 comparison*).tw,kf,kw.	596
69	(multi* adj2 paramet* adj2 evidence adj2 synthesis).tw,kf,kw.	35
70	(multiparamet* adj2 evidence adj2 synthesis).tw,kf,kw.	35
71	(multi-paramet* adj2 evidence adj2 synthesis).tw,kf,kw.	29
72	or/57-71	1023177
73	13 or 30	8244
74	(13 or 30) not (56 or 72)	7679
75	limit 74 to (english or french or german or italian)	6913
76	limit 75 to yr="1860 - 2010"	4339
77	remove duplicates from 76	3566
78	limit 75 to yr="2011 -Current"	2573
79	remove duplicates from 78	2031
80	77 or 79	5597
81	31 or 46	4301
82	(31 or 46) not (56 or 72)	3949
83	limit 82 to (english or french or german or italian)	3650
84	remove duplicates from 83	2887
85	80 or 84	6339

Table 31 Search strategy for primary studies in the Cochrane Library (4 November 2020)

#	Search terms	Results
1	MeSH descriptor: [Vitamin B 12 Deficiency] explode all trees	152
2	(B12 OR B-12 OR "b(12)" OR AdCbl OR AdoCbl):ti AND (deficien* OR inadequa* OR insufficien*):ti	61
3	(adenosylcobalamin? OR avitaminos#s OR Cbl OR CN-Cbl OR cobalamin? OR coballamin? OR cobamides OR cyanocobalamin?):ti AND (deficien* OR inadequa* OR insufficien*):ti	18
4	(deoxyadenosinecobalamin? OR eritron OR hydroxocobalamin? OR methylcobalamin?):ti AND (deficien* OR inadequa* OR insufficien*):ti	1
5	#1 OR #2 OR #3 OR #4 in Trials	192

Table 32 Search strategy – Speciality society websites (4 November 2020)

Society	Website	Search terms		
		Vitamin B12	Cobalamin	Total relevant
Australasian Society for Parenteral and Enteral Nutrition	www.auspen.org.au	-	-	-
Flemish Society for Clinical Nutrition and Metabolism Vlaamse Vereniging voor Klinische Voeding en Metabolisme	www.vvkvm.be	-	-	-
Société Belge de Nutrition Clinique Belgian Society of Clinical Nutrition	www.sbnc.be	-	-	-
Canadian Nutrition Society Société Canadienne de Nutrition	www.cns-scn.ca	0	0	0
Hrvatsko društvo za klinicku prehranu, Hrvatskog liječničkog zbora Croatian Society of Clinical Nutrition Croatian Medical Association	www.hlz.hr	0	0	0
Česká společnost klinické výživy a intenzivní metabolické péče České lékařské společnosti Jana Evangelisty Purkyně The Czech Society for Clinical Nutrition and Intensive Metabolic Care within the Czech Medical Association of J.E. Purkyne	www.skvimp.cz	0	0	0
Dansk Selskab for Klinisk Ernæring Danish Society for Clinical Nutrition	www.dske.dk	-	-	-
The Finnish Society for Clinical Nutrition and Metabolism	www.fispen.fi	-	-	-
Société Francophone Nutrition Clinique et Métabolisme French Speaking Society of Clinical Nutrition and Metabolism	www.sfncm.org	0	0	0
Deutsche Gesellschaft für Ernährungsmedizin German Society for Nutritional Medicine	www.dgem.de	0	0	0
Ελληνική Εταιρεία Κλινικής Διατροφής και Μεταβολισμού Hellenic Society for Clinical Nutrition and Metabolism	www.grespen.org	0	0	0
Irish Society for Clinical Nutrition and Metabolism	www.irspen.ie	0	0	0
Hachevra Letzona Clinit The Israeli Society for Clinical Nutrition	www.ima.org.il	0	0	0
Societa Italiana di Nutrizione Artificiale e Metabolismo Italian Society for Artificial Nutrition and Metabolism	www.sinpe.org	0	0	0
日本静脈経腸栄養学会 Japanese Society for Parenteral and Enteral Nutrition	www.jspen.jp/top.html	0	0	0
Norsk Selskap for Klinisk Ernæring Norwegian Society for Clinical Nutrition and Metabolism	www.nske.no	-	-	-
Associação Portuguesa de Nutrição Entérica e Parentérica Portuguese Association of Enteral and Parenteral Nutrition	www.apnep.pt	-	-	-
Society for Parenteral and Enteral Nutrition (Singapore)	www.singspen.org.sg	-	-	-
Slovensko Združenje za Klinično Prehrano	http://klinicnaprehra	0	0	0

Society	Website	Search terms		
		Vitamin B12	Cobalamin	Total relevant
Slovenian Society for Clinical Nutrition	na.si/			
Sociedad Española de Nutrición Enteral y Parenteral Spanish Society for Parenteral and Enteral Nutrition	www.senpe.com	1 (consensus document)	0	1
The Swedish Society for Clinical Nutrition and Metabolism	www.swespen.se	-	-	-
Gesellschaft für Klinische Ernährung der Schweiz / Société Suisse de Nutrition Clinique Swiss Society for Clinical Nutrition	www.geskes.ch	0	0	0
Netherlands Society for Parenteral and Enteral Nutrition van de Nederlandse Vereniging voor Gastro-Enterologie	www.nvge.nl/	-	-	-
British Association of Parenteral and Enteral Nutrition	www.bapen.org.uk	0	0	0
American Society for Parenteral and Enteral Nutrition	www.nutritioncare.org	1 (recommendation not HTA)	1 (same document)	1
Geriatric				
European Geriatric Medicine Society	https://www.eugms.org/home.html	X	X	X
Australia and New Zealand Society for Geriatric Medicine	http://www.anzsgm.org/	0	0	0
Swiss Geriatric Society / Schweizerische Fachgesellschaft für Geriatrie)	https://www.sfgg.ch/	0	0	0
Haematology				
Belgian Hematology Society	http://www.bhs.be	0	0	0
Cyprus Society of Haematology	www.cyhaema.com	-	-	-
Ceská Hematologická Spolecnost CLS JEP (Czech Society of Hematology)	http://www.hematology.cz	0	0	0
Dansk Haematologisk Selskab (Danish Society of Hematology)	http://www.hematology.dk	0	0	0
Helsinki University Central Hospital Cancer Center	www.hematology.fi/en	0	0	0
Société Française d'Hématologie (French Society of Hematology)	http://sfh.hematologie.net	0	0	0
Deutsche Gesellschaft für Hämatologie und Onkologie (German Society of Hematology)	http://www.dgho.de	0	0	0
Eliniki Ematologhiki Eteria (Hellenic Society of Hematology)	http://www.eae.gr	0	0	0
Haematology Association of Ireland	http://www.haematologyireland.org	0	0	0
Israel Society of Hematology and Blood Transfusion	http://www.hematology.org.il	-	-	-
Società Italiana di Ematologia (Italian Society of Hematology)	http://www.siematologia.it	0	0	0
Société Luxembourgoise d'Oncologie	https://www.slo.lu	-	-	-
Sociedade Portuguese de Hematologia (Portugese Society of Hematology)	http://www.sph.org.pt	0	0	0
Zdruzenja Hematologov Slovenije (Slovenian Society of Hematology)	http://www.hematologija.org	-	-	-

Society	Website	Search terms		
		Vitamin B12	Cobalamin	Total relevant
Sociedad Española de Hematología y Hemoterapia	http://sehh.es/es/	0	0	0
Swedish Society of Hematology	http://www.sfhem.se	0	0	0
Schweizerische Gesellschaft für Hämatologie / Société Suisse d'Hématologie (Swiss Society for Hematology)	http://www.sgh-ssh.ch	0	0	0
Nederlandse Vereniging voor Hematologie (Dutch Society of Hematology)	https://www.hematologienederland.nl	0	0	0
British Society for Haematology	http://www.b-s-h.org.uk	0	1	1
Neurology				
Brain Research Society of Finland	https://www.brsf.org/	-	-	-
British Neuroscience Association	https://www.bna.org.uk/	-	-	-
Croatian Society for Neuroscience	http://www.hiim.unizg.hr/	-	-	-
Czech Neuroscience Society	http://www.biomed.cas.cz/cns/indexEN.html	-	-	-
Danish Society for Neuroscience	https://dsfn.dk/	-	-	-
Dutch Neurofederation	https://neurofederatie.nl/	-	-	-
German Neuroscience Society	https://nwg-info.de/	-	-	-
Hellenic Society for Neuroscience	https://www.hsfn.gr/	-	-	-
Israel Society for Neuroscience	https://www.isfn.org.il/	-	-	-
Italian Society for Neuroscience	http://www.sins.it/EN/index.xhtml	0	0	0
Malta Neuroscience Network	http://mnn.mt/	-	-	-
Neuroscience Ireland	https://neuroscienceireland.com/	-	-	-
Norwegian Neuroscience Society	https://www.ntnu.edu/nns	-	-	-
Slovenian Neuroscience Association	http://www.sinapsa.org/naslovnica/	-	-	-
Sociedad Española de Neurociencia	https://www.senc.es/en/	0	0	0
Sociedade Portuguesa de Neurociências	http://www.spn.org.pt/	0	0	0
Societé des Neurosciences	https://www.neurosciences.asso.fr/	-	-	-
Swiss Society for Neuroscience	https://www.swissneuroscience.ch/	-	-	-

Abbreviations
HTA = health technology assessment, **-** = search unavailable, **X** = issue accessing website or with website function.

Table 33 HTA agency literature search, 4 November 2020

HTA agency	Source	Search terms		Total relevant
		Vitamin B12	Cobalamin	
International				
International Information Network on New and Emerging Health Technologies (EuroScan International Network)	https://www.euroscan-network.global/index.php/en/47-public-features/761-database-home	0	0	0
Australia				
Adelaide Health Technology Assessment (AHTA)	https://www.adelaide.edu.au/ahta/pubs/	X	X	X
Australian Safety and Efficacy Register of New Interventional Procedures—Surgical (ASERNIP-S)	https://www.surgeons.org/research-audit/research-evaluation-inc-asernips	0	0	0
Australia & New Zealand				
Health Technology Reference Group (HTRG)	https://www.coaghealthcouncil.gov.au/AHMAC/Health-Technology-Reference-Group	0	0	0
Austria				
Austrian Institute of Technology Assessment (AIHTA)	https://www.oeaw.ac.at/ita/publikationen/	0	0	0
Belgium				
Belgian Health Care Knowledge Centre (KCE)	http://kce.fgov.be	0	0	0
Canada				
Institute of Health Economics (IHE)	http://www.ihe.ca	0	0	0
Institut National d'Excellence en Santé et en Services (INESSS)	https://www.inesss.qc.ca/en/home.html	0	0	0
The Canadian Agency for Drugs and Technologies in Health (CADTH)	http://www.cadth.ca/	3	3	3 (duplicates)
Evidence Development and Standards Branch (HQO)	http://www.hqontario.ca	3	2	3(duplicates)
Denmark				
Social & Health Services and Labour Market (DEFACTUM)	http://www.defactum.net	0	0	0
Finland				
Finnish Coordinating Center for Health Technology Assessment (FinCCHTA)	https://www.ppshp.fi/Tutkimus-ja-opetus/FinCCHTA/Sivut/HTA-julkaisuja.aspx	0	0	0
Finnish Medicines Agency (FIMEA)	http://www.fimea.fi	0	0	0
France				
French National Authority for Health (Haute Autorité de Santé; HAS)	http://www.has-sante.fr/	0	0	0
Comité d'Evaluation et de Diffusion des Innovations Technologiques (CEDIT)	http://cedit.aphp.fr/	0	0	0
Germany				
Institute for Quality and Efficiency in Health Care (IQWiG)	http://www.iqwig.de	0	0	0

Federal Joint Committee (Gemeinsamer Bundesausschuss; G-BA)	https://www.g-ba.de/english/	0	0	0
Ireland				
Health Information and Quality Authority (HIQA)	http://www.hiqa.ie	0	0	0
Italy				
Agenzia Sanitaria e Sociale Regionale (ASSR)	http://www.inahta.org/members/assr/	0	0	0
HTA Unit in A. Gemelli Teaching Hospital (UVT)	https://www.policlinicogemelli.it/	X	X	X
National Agency for Regional Health services (Agenas)	http://www.agenas.it	-	-	-
The Netherlands				
The Netherlands Organisation for Health Research and Development (ZonMw)	http://www.zonmw.nl	0	0	0
Zorginstituut Nederland (ZIN)	https://www.zorginstituutnederland.nl/	0	0	0
Norway				
The Norwegian Institute of Public Health (NIPH)	http://www.fhi.no/	0	0	0
Singapore				
Agency for Care Effectiveness (ACE)	http://www.ace-hta.gov.sg/	0	0	0
Spain				
Agencia de Evaluación de Tecnologias Sanitarias, Instituto de Salud “Carlos III”I / Health Technology Assessment Agency (AETS)	http://publicaciones.isciii.es/	0	0	0
Agency for Health Quality and Assessment of Catalonia (AQuAS)	http://aquas.gencat.cat	0	0	0
Andalusian HTA Agency (AETSA)	http://www.aetsa.org/	0	0	0
Basque Office for Health Technology Assessment (OSTEBA)	http://www.euskadi.eus/web01-a2ikeost/en/	0	0	0
Galician Agency for Health Technology Assessment (AVALIA-T)	http://acis.sergas.es	0	0	0
Health Sciences Institute in Aragon (IACS)	http://www.iacs.es/	0	0	0
Sweden				
Swedish Council on Technology Assessment in Health Care (SBU)	http://www.sbu.se/en/	0	0	0
Switzerland				
Swiss Federal Office of Public Health (SFOPH)	http://www.bag.admin.ch/hta	0	0	0
United Kingdom				
Healthcare Improvement Scotland (HIS)	http://www.healthcareimprovementscotland.org	0	0	0
NHS Quality Improvement Scotland	http://www.nhshealthquality.org/	0	0	0
National Institute for Clinical Excellence (NICE)	http://www.nice.org.uk/	1 (recommendation)	1	0
Health Technology Wales (HTW)	http://www.healthtechnology.wales	0	0	0

National Institute for Health Research (NIHR), including HTA programme	http://www.nets.nihr.ac.uk/programmes/hta	0	0	0
United States				
Agency for Healthcare Research and Quality (AHRQ)	https://www.ahrq.gov/research/findings/index.html	0	0	0

Abbreviations
HTA = health technology assessment, **(-):** search unavailable; **X:** issue accessing website or with website function
***Source**: Based on registered INAHTA agencies located in *WHO-Mortality Stratum A* countries.

Additional Legal, Social, Ethical and Organisational Searches

Table 34 Search string for legal issues (25 November 2020)

#	Query	Medline	Embase
1	personal autonomy.sh.	17214	14263
2	Human rights.sh.	14493	267105
3	Human rights.ti,ab.	8598	32374
4	free will.mp.	746	932
5	self determination.mp.	4935	5754
6	Parental consent.sh.	3268	5222
7	Third-party consent.sh.	3760	45
8	Presumed consent.sh.	549	435
9	Informed consent by minors.sh.	220	13
10	Consent.ti,ab.	63990	131775
11	Privacy.mp.	21378	27620
12	Confidentiality.sh.	23520	33490
13	Confidentiality.ti,ab.	11358	14046
14	Personally identifiable information.sh.	44	71
15	health records.mp.	30378	18565
16	personal information.mp.	2054	2893
17	Jurisprudence.sh.	29803	55878
18	Law enforcement.sh.	3775	14010
19	Law.ti,ab.	90201	104059
20	Laws.ti,ab.	30110	33117
21	Legislation, Drug/	10300	15749
22	Legislation, Medical/	16557	146
23	Legislation as Topic/	15938	80362
24	Legislation, Food/	2439	166
25	Legislation, Hospital/	2449	44
26	Legislation, Pharmacy/	1253	158
27	legislation.ti,ab.	33286	43360
28	Civil rights.sh.	10023	9740
29	Authority.ti,ab.	22733	30377
30	Legal case.pt.	11029	489
31	Legal guardians.sh.	2057	995

#	Query	Medline	Embase
32	Legal.ti,ab.	76220	105594
33	Liability, legal.sh.	15696	16948
34	Legal services.sh.	31	653
35	Access to information.sh.	6590	22242
36	Social justice.sh.	12289	13070
37	Health equity.sh.	1439	11819
38	Human rights abuses.sh.	817	1626
39	Patient rights.sh.	7073	13986
40	Rights to human.sh.	0	8
41	Ownership.sh.	8982	16388
42	Intellectual property.sh.	1577	4093
43	Intellectual property.ti,ab.	2149	3051
44	Licensure.sh.	7098	35143
45	License.ti,ab.	7860	12239
46	Liability, legal.sh.	15696	16948
47	Liability.ti,ab.	19165	24109
48	Legislation.sh.	1670	80362
49	Legislation/	1670	80362
50	Medical device legislation.sh.	250	1011
51	Conflict of interest.sh.	9873	16213
52	Guaranty.ti,ab.	124	234
53	Regulation.ti,ab.	850766	1039558
54	Acquisition.mp.	155865	202161
55	Conflict of interest.ti,ab.	3954	6189
56	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42 or 43 or 44 or 45 or 46 or 47 or 48 or 49 or 50 or 51 or 52 or 53 or 54 or 55	1491377	2096898
57	exp *Vitamin B 12 Deficiency/	8277	10955
58	Vitamin B 12/df	0	13116
59	Cobamides/df	17	1457
60	Hydroxocobalamin/df	1	2829
61	((((vitamin* or hypovitaminosis or hypo-vitaminosis) adj (B12 or B-12 or "b(12)")) or AdCbl or AdoCbl or adenosylcobalamin or avitaminosis or Cbl or CN-Cbl or cobalamin? or coballamin? or cobamides or cyanocobalamin? or deoxyadenosinecobalamin? or eritron or hydroxocobalamin? or hydroxo-cobalamin? or MeCbl or methylcobalamin? or methyl-cobalamin?) adj2 (deficien* or inadequa* or insufficien*)).ti,kf,kw.	2736	37448
62	((B12 or B-12 or "b(12)" or AdCbl or AdoCbl or adenosylcobalamin or avitaminosis or Cbl or CN-Cbl or cobalamin? or coballamin? or cobamides or cyanocobalamin? or deoxyadenosinecobalamin? or eritron or hydroxocobalamin? or hydroxo-cobalamin? or MeCbl or methylcobalamin? or methyl-cobalamin?) and (deficien* or inadequa* or insufficien*)).ti.	2958	19023
63	((pernicious or Addison*) adj anemi*).ti,kf,kw.	2197	8249
64	Vitamin B 12.mf	17843	23350
65	57 or 58 or 59 or 60 or 61 or 62 or 63 or 64	22868	51743
66	56 and 65 (Vitamin B12 deficiency and legal)	376	2252

#	Query	Medline	Embase
67	66 and conference abstract, papers or review	0	639
68	66 not 67	376	1613

Table 35 Search string for social issues (24 November 2020)

#	Query	Medline	Embase
1	Patient experience.ti,ab.	5791	396933
2	Quality of life.sh.	200179	599332
3	Social aspects of.ti,ab.	3785	75413
4	Medical decision-making process.mp.	148	203
5	Patient Education as Topic/	85879	126835
6	Patient education.ti,ab.	17933	27380
7	Patient attitude.ti,ab.	144	205
8	Patient preference.ti,ab.	4099	6963
9	Patient decision.ti,ab.	1670	2662
10	Patient acceptance.ti,ab.	2816	3886
11	Patient satisfaction.ti,ab.	36477	52836
12	Patient-focused.ti,ab.	1621	2326
13	Patient-centred.ti,ab.	5732	8234
14	Patient advocacy.ti,ab.	1343	2054
15	Consumer satisfaction.ti,ab.	739	889
16	Consumer participation.ti,ab.	307	380
17	Consumer preference.ti,ab.	388	403
18	Consumer attitude.ti,ab.	39	50
19	Self-perception.mp.	4572	5988
20	Self-care.mp.	44945	68082
21	Self-efficacy.mp.	37487	35582
22	Attitude to health.mp.	199949	116449
23	Health education.mp.	88519	149087
24	Health knowledge.mp.	115813	4664
25	Informed choice.mp.	1592	2073
26	Shared decision making.mp.	9080	15206
27	Empowerment.mp.	13107	20265
28	Quality of Life.mp.	356571	599332
29	Adaptation, psychological.mp.	95504	60891
30	Coping.mp.	56311	97357
31	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30	984356	1568154
32	exp *Vitamin B 12 Deficiency/	8277	10955
33	Vitamin B 12/df	0	13116
34	Cobamides/df	17	1457
35	Hydroxocobalamin/df	1	2829
36	((((vitamin* or hypovitaminosis or hypo-vitaminosis) adj (B12 or B-12 or "b(12)")) or AdCbl	2736	37448

#	Query	Medline	Embase
	or AdoCbl or adenosylcobalamin or avitaminosis or Cbl or CN-Cbl or cobalamin? or coballamin? or cobamides or cyanocobalamin? or deoxyadenosinecobalamin? or eritron or hydroxocobalamin? or hydroxo-cobalamin? or MeCbl or methylcobalamin? or methyl-cobalamin?) adj2 (deficien* or inadequa* or insufficien*)).ti,kf,kw.		
37	((B12 or B-12 or "b(12)" or AdCbl or AdoCbl or adenosylcobalamin or avitaminosis or Cbl or CN-Cbl or cobalamin? or coballamin? or cobamides or cyanocobalamin? or deoxyadenosinecobalamin? or eritron or hydroxocobalamin? or hydroxo-cobalamin? or MeCbl or methylcobalamin? or methyl-cobalamin?) and (deficien* or inadequa* or insufficien*)).ti.	2958	19023
38	((pernicious or Addison*) adj anemi*).ti,kf,kw.	2197	8249
39	Vitamin B 12.mf	17843	23350
40	32 or 33 or 34 or 35 or 36 or 37 or 38 or 39	22868	51743
41	31 and 40 (Vitamin B12 deficiency and social)	254	1286
42	41 and conference abstract, paper or review	0	487
43	41 not 42	254	799

Table 36 Search string for ethical issues (25 November 2020)

#	Query	Medline	Embase
1	Ethics.sh.	9768	251760
2	Medical ethics.ti,ab.	5617	6765
3	Ethical theory.ti,ab.	288	302
4	Bioethics.ti,ab.	6711	7825
5	Bioethics.sh.	7933	40366
6	Morals.sh.	15665	838
7	Morality.ti,ab.	4199	5031
8	Principle-based ethics.sh.	892	47
9	Patient rights.sh.	7073	13986
10	Patient autonomy.ti,ab.	2328	2863
11	Personal autonomy.sh.	17214	14239
12	Autonomy.ti,ab.	29626	36908
13	Social justice.sh.	12289	13070
14	Ethical issues.ti,ab.	11987	14381
15	Normative.ti,ab.	29968	38290
16	Social Stigma/	8811	10059
17	Stigma*.mp.	40591	56905
18	Psychological Distress/	1041	26446
19	Discrimin*.mp.	298651	386492
20	confidentiality.mp.	29668	33490
21	privacy.mp.	21378	27620
22	Informed Consent/	36724	132625
23	Confidentiality/	23520	33490
24	Privacy/	6587	27620
25	overdiagnosis.mp.	3457	6141
26	underdiagnosis.mp.	2043	4133

#	Query	Medline	Embase
27	beneficence.mp.	4627	5109
28	non-maleficence.mp.	516	687
29	vulnerable groups.mp.	2903	3482
30	Health Equity/	1439	11819
31	health equity.mp.	4510	27651
32	utilitarian*.mp.	1804	2017
33	Non-consequentialist.mp.	15	14
34	Respect/	372	443010
35	dignity.mp.	7057	10375
36	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35	545546	1403403
37	exp *Vitamin B 12 Deficiency/	8277	10955
38	Vitamin B 12/df	0	13116
39	Cobamides/df	17	1457
40	Hydroxocobalamin/df	1	2829
41	((((vitamin* or hypovitaminosis or hypo-vitaminosis) adj (B12 or B-12 or "b(12)")) or AdCbl or AdoCbl or adenosylcobalamin or avitaminosis or Cbl or CN-Cbl or cobalamin? or coballamin? or cobamides or cyanocobalamin? or deoxyadenosinecobalamin? or eritron or hydroxocobalamin? or hydroxo-cobalamin? or MeCbl or methylcobalamin? or methyl-cobalamin?) adj2 (deficien* or inadequa* or insufficien*)).ti,kf,kw.	2736	37448
42	((B12 or B-12 or "b(12)" or AdCbl or AdoCbl or adenosylcobalamin or avitaminosis or Cbl or CN-Cbl or cobalamin? or coballamin? or cobamides or cyanocobalamin? or deoxyadenosinecobalamin? or eritron or hydroxocobalamin? or hydroxo-cobalamin? or MeCbl or methylcobalamin? or methyl-cobalamin?) and (deficien* or inadequa* or insufficien*)).ti.	2958	19023
43	((pernicious or Addison*) adj anemi*).ti,kf,kw.	2197	8249
44	Vitamin B 12.mf	17843	23350
45	37 or 38 or 39 or 40 or 41 or 42 or 43 or 44	22868	51743
46	36 and 45 (Vitamin B12 deficiency and ethical)	118	1187
47	46 and conference abstract, paper or review	0	799
48	46 not 47	118	423

Table 37 Search string for organisational issues (25 November 2020)

#	Query	Medline	Embase
1	(Information storage and retrieval).mp.	20559	561
2	information management.mp.	8123	16005
3	Health information systems.sh.	1329	2743
4	Health information management.sh.	840	21688
5	Health information exchange.sh.	902	1145
6	Information literacy.sh.	262	849
7	Health equity.sh.	1439	11819
8	work process.mp.	968	1139
9	work flow.mp.	1411	39056

#	Query	Medline	Embase
10	Medical Education.sh.	0	327136
11	Education, Professional, Retraining/	1242	8
12	Education, public health professional.sh.	789	7
13	Health information interoperability.sh.	173	52
14	Communication.sh.	85223	616551
15	Health communication.sh.	2484	8227
16	Quality assurance, health care.sh.	56106	174
17	Implementation science.sh.	528	5920
18	human skills.mp.	82	95
19	Organizational Culture/	17730	3590
20	Sustainability.ti,ab.	24103	29660
21	system structure.mp.	761	874
22	Acceptance.ti,ab.	70692	94684
23	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 1 or 16 or 17 or 18 or 19 or 20 or 21 or 22	286820	1124220
24	exp *Vitamin B 12 Deficiency/	8277	10955
25	Vitamin B 12/df	0	13116
26	Cobamides/df	17	1457
27	Hydroxocobalamin/df	1	2829
28	((((vitamin* or hypovitaminosis or hypo-vitaminosis) adj (B12 or B-12 or "b(12)")) or AdCbl or AdoCbl or adenosylcobalamin or avitaminosis or Cbl or CN-Cbl or cobalamin? or coballamin? or cobamides or cyanocobalamin? or deoxyadenosinecobalamin? or eritron or hydroxocobalamin? or hydroxo-cobalamin? or MeCbl or methylcobalamin? or methyl-cobalamin?) adj2 (deficien* or inadequa* or insufficien*)).ti,kf,kw.	2736	37448
29	((B12 or B-12 or "b(12)" or AdCbl or AdoCbl or adenosylcobalamin or avitaminosis or Cbl or CN-Cbl or cobalamin? or coballamin? or cobamides or cyanocobalamin? or deoxyadenosinecobalamin? or eritron or hydroxocobalamin? or hydroxo-cobalamin? or MeCbl or methylcobalamin? or methyl-cobalamin?) and (deficien* or inadequa* or insufficien*)).ti.	2958	19023
30	((pernicious or Addison*) adj anemi*).ti,kf,kw.	2197	8249
31	Vitamin B 12.mf	17843	23350
32	24 or 25 or 26 or 27 or 28 or 29 or 30 or 31	22868	51743
33	23 and 32 (Vitamin B12 deficiency and organisational)	38	627
34	33 and conference abstracts, paper or review	0	116
35	33 not 34	38	511

Appendix B: Evidence tables

12.1 Evidence tables for the linked evidence, systematic reviews and HTAs

Table 38 Characteristics of systematic reviews and HTAs evaluating diagnostic accuracy

Author year	Databases searched & end search date	Inclusion criteria	Included studies	Conclusions
Australia Government Department of Health 2014[190] (HTA)	OVID, Medline, PubMed, The Cochrane Library & relevant HTA websites and databases – all searched to September 2013	P: Patients at risk of B12 deficiency I: B12 testing C: No B12 testing O: Safety; patient outcomes including morbidity, mortality, QOL Studies: 2006-2013 English only, SRs, primary studies if prospective & comparative	Only one relevant document was located – a rapid review by Health Quality Ontario 2012[193] that included 54 studies plus a meta-analysis by Willis 2011[34]	There are no recommendations on the frequency of vitamin B12 testing and there is no direct evidence regarding the clinical utility of vitamin B12 testing in any population.
Willis, 2011[34]	PubMed, EMBASE, CINAHL/PsycINFO, Web of Science, Current Contents & Informit Health Databases1990 to November 2009	P: any patient presentations (e.g. suspected anaemia, dementia) in diverse settings (e.g. general practice, hospital in-patients) I: B12 test C: MMA, Hcy, holoTC or any other clinical reference standard O: Diagnostic accuracy of serum B12 tests RCTs, diagnostic cohort, case-control, therapeutic trial, nested case-control & cross-sectional studies	54 studies of various designs contributed 107 data sets. Through reporting or calculation, 75 2x2 data sets were obtained for analysis. 36 studies were included in a meta-analysis.	Diagnosis of conditions amenable to B12 supplementation on the basis of serum B12 level alone cannot be considered a reliable approach to investigating suspected B12 deficiency

Abbreviations
B12 = vitamin B12, **holoTC** = holotranscobalamin II, **Hcy** = homocysteine, **HTA** = health technology assessment, **MMA** = methylmalonic acid, **PICO** = population, intervention, comparator and outcome, **QOL** = quality of life, **RCT** = randomised controlled trial, **SR** = systematic review.

Table 39 Characteristics of the systematic review evaluating change in management

Author year	Databases searched & end search date	Inclusion criteria	Included studies	Conclusions
Hvas 2003[191] (HTA)	Medline, Embase & Current Contents 1986 to 2001	P: Medical records of patients with elevated MMA (>28 µmol/L) at 3 district hospitals and 10 GPs I: None C: Recommendations O: physicians' reasons for requesting MMA & their reaction to increased levels of MMA Included studies relating to the clinical usefulness of MMA and heavily relying on two earlier primary studies by their research group (Hvas 2000 & Hvas 2002)[59 60]	For changes in management, no published studies were used, rather the authors performed chart reviews and gathered primary data.	In the decade following the introduction of MMA testing, there was no decrease in orders for serum B12 levels. Hospitals: Ignored in 62% of cases (n = 109) and the recommendation to treat (when MMA >0.45 µmol/L) was rarely followed. GP offices: Recorded in 71% of cases and vitamin B12 treatment was initiated in 80% of these patients.

Abbreviations
B12 = vitamin B12, **GP** = general practitioner, **HTA** = health technology assessment, **MMA** = methylmalonic acid, **µmol/L** = micromoles per litre.

Table 40 Characteristics of systematic reviews evaluating the effectiveness of supplementation

Author year	Databases searched & end search date	Inclusion criteria	Included studies	Conclusions
Andres 2018[118]	PubMed & Google Scholar January 2010 to June 2018; The Cochrane Library & the ISIWeb of Knowledge (dates not provided)	P: Adults with B12 deficiency related to a GI disorder, malnutrition or malabsorption (including veganism or vegetarianism & surgery) I: B12 oral or nasal vs IM C: Unclear – not required O: Change in serum B12 levels Articles in English & French; clinical trials, review articles & guidelines	39 studies included Oral therapy: 4 RCTs (oral vs IM), 4 SRs, 6 narrative reviews, 13 prospective observational studies, all concerning patients with a specific disorder or disease Oral therapy included sublingual in some cases Nasal therapy: 1 preliminary study	Oral vitamin B12 is an effective alternative to IM dosing, except in patients with severe neurological manifestations. It avoids the discomfort, contraindication (in patients with anticoagulation) & cost of monthly injections. More research is needed on nasal administration,

Author year	Databases searched & end search date	Inclusion criteria	Included studies	Conclusions
Butler 2006[119] (distillation of a Cochrane review)	Medline, Embase, The Cochrane library, DARE database & Lilacs December 2004	P: People with low B12 levels (threshold: 180 pmol/L) I: Oral B12 treatment C: IM B12 treatment O: Serum vitamin B12, Hcy or MMA levels; MCV; clinical signs & symptoms of B12 deficiency; costs; AEs; acceptability to patients & QOL RCTs, published or unpublished; no language restrictions	2 small RCTs of oral versus IM therapy included (Turkey & USA) that reported outcomes for 60 & 33 patients, respectively)	The evidence derived from limited studies suggests that high oral doses of B12 (1,000 µg and 2,000 µg) could be as effective as IM administration in achieving haematological and neurological responses. The study was limited by the short follow up period of 90 and 120 days in the included RCTs.
Chan 2016[19]	PubMed March 31, 2016	P: People with pernicious anaemia I: Oral B12 therapy C: Unclear – IM B12 in some studies O: Serum B12 levels in response to B12 therapy English language only	12 articles: 2 RCTs, 3 prospective studies, 1 SR (Butler 2008) & 6 clinical reviews	"Oral B12 is an effective alternative to vitamin B12 IM" "Patients should be offered the [oral] alternative after an informed discussion." Patients beginning oral therapy should be monitored closely for normalisation of vitamin B12 levels and symptoms. Hcy and MMA testing costs, availability of tests, and issues with surrounding reference values are should be considered in regards to monitoring after supplementation.
Mahawar 2018[120]	PubMed September 22, 2017	P: Adult patients who had undergone proximal RYGB (weight-loss surgery) I: Oral B12 therapy – various doses C: None required O: Prevention of B12 deficiency English language only	19 articles: 6 comparative, 11 cohort, 2 RCTs	"Oral supplementation doses ≤ 15 µg vitamin B12 daily are inadequate for prophylaxis of B12 deficiency in adult RYGB patients but doses of 1,000 µg daily might be adequate."

Author year	Databases searched & end search date	Inclusion criteria	Included studies	Conclusions
Moore 2012[32]	Medline, PubMed, PsychINFO March 2011	P: People with neurodegenerative disease &/or CI I: Serum B12 levels C: NA O: Diagnosis of neurodegenerative disease &/or CI English language & published after 1995	43 studies on the association of vitamin B12 & CI or dementia. 17 reported on the efficacy of B12 therapy for these conditions. 6 case reports, 43 observational/case-control, 8 intervention studies, 9 placebo-controlled studies, 8 meta-analysis/literature reviews, 4 invitro/cell culture and in vivo	"Low serum vitamin B12 levels are associated with neurodegenerative disease & CI" (<250 pmol/L and <150 pmol/L respectively). A small subset of dementia cases are reversible with B12 high dose therapy. Vitamin B12 therapy is safe and inexpensive.
Smelt 2017[122]	PubMed, Embase, Medline & The Cochrane Library December 2015	P: Patients before or after bariatric surgery I: B12 supplementation C: No B12 supplementation or different supplementation regimen O: Vitamin B12 levels RCT or cohort study, all types of bariatric surgery	10 studies (n= 1,277 participants):. 1 RCT, 4 prospective cohort studies, 5 retrospective cohort studies Quality (using the Newcastle-Ottawa scale) ranged from moderate to good.	"The current literature suggests that 350 µg oral vitamin B12 (likely life-long) is the appropriate dose to correct low vitamin B12 levels in many patients."
Smelt 2018[121]	PubMed, EMBASE, Web of Science, Cochrane, CENTRAL, ClinicalTrials.gov April 2016 .	P: Community-dwelling adults aged 60+ years I: B12 or folic acid supplementation C: Placebo O: Changes in haemoglobin, B12, MMA, Hcy, s-folate, MCV, Hct & RBC count	10 RCTs that reported on 4 unique studies using individual participant data: published from 2001 to 2011; sample sizes 40 to 155 people (total 343); therapy was oral or IM; conducted in Australia, Denmark, Switzerland, England.	Evidence of the impact of vitamin B12 on haematological parameters was inconclusive; further research is needed before firm recommendations on supplementation can be made

Author year	Databases searched & end search date	Inclusion criteria	Included studies	Conclusions
Wang 2018[8] Cochrane review	CENTRAL, MEDLINE, Embase, LILACS, WHO ICTRP, ClinicalTrials.gov 17 July 2017	P: B12 deficient people, i.e. low serum B12 levels I: Oral B12 C: IM B12 O: Serum vitamin B12 levels, signs & symptoms of B12 deficiency, QOL, AEs, acceptability to patients, haemoglobin and mean corpuscular volume, total Hcy and serum methylmalonic acid levels, and socioeconomic effects. RCTs, no language restrictions	3 RCTs – total enrolled 153 people (74 to oral B12, 79 to IM). Treatment duration and follow-up 3-4 months	Low quality evidence (few trials, all small) showed that oral and IM vitamin B12 had similar efficacy in terms of normalising serum vitamin B12 levels but oral treatment was lower cost. Very low quality studies suggested that safety was equivalent between IM and oral routes. No RCT reported on clinical signs and symptoms of vitamin B12 deficiency, QOL, or acceptability of treatment scheme.

Abbreviations

AE = adverse effect, **B12** = vitamin B12, **CI** = cognitive impairment, **GI** = gastrointestinal, **Hct** = haematocrit, **Hcy** = homocysteine, **ICTRP** = International Clinical Trials Registry Platform, **IM** = intramuscular, **MCV** = mean corpuscular volume, **MMA** = methylmalonic acid, **NA** = not applicable, **PICO** = population, intervention, comparator and outcome, **pmol/L** = picomoles per litre, **QOL** = quality of life, **RBC** = red blood cell, **RCT** = randomised controlled trial, **RYGB** = Roux-en-Y gastric bypass, **s-** = serum, **SR** = systematic review, **USA** = United States of America, **WHO** = World Health Organization, **µg** = micrograms.

Table 41 Characteristics of systematic reviews evaluating the natural course of vitamin B12 deficiency

Author year	Databases searched and search date	Inclusion criteria	Included studies	Conclusions
Moore 2012[32]	Medline, PubMed, PsychINFO March 2011	P: People with neurodegenerative disease /or CI I: Serum B12 levels C: NA O: Diagnosis of neurodegenerative disease &/or CI English language & published after 1995	43 studies investigated the association of vitamin B12 & CI or dementia. 6 case reports, 43 observational/case-control, 8 intervention studies, 9 placebo-controlled studies, 8 meta-analysis/literature reviews, 4 invitro/cell culture and in vivo	*"B12 levels in the subclinical low-normal range (<250pmol/L) are associated with Alzheimer's disease, vascular dementia & Parkinson's disease. Vegetarianism & metformin use contribute to low levels & may independently increase CI risk. Vitamin B12 deficiency (<150 pmol/L) is associated with CI*."
Petridou 2016[123]	Medline June 2013	P: Older people with low B12 levels I: NA C: NA O: Odds ratios for depression (using various scales) & impact of sex No language restrictions	9 studies (n = 6,308 people); 1989 to 2013; 1 cohort, 1 case-control, 7 cross-sectional design; people generally age ≥65 but ≥55 in 2 studies; 3 studies reported data by sex	In a meta-analysis, "*[low] B12 serum levels seem to be associated with depression in the aged*". Sex-specific analysis via meta-analysis showed a positive association of low B12 with depression among older women, primarily based on the results of the largest study.
Ray 2003[124]	Medline October 2002	P: Women with a prior or current NTD-affected pregnancy I: Serum or plasma B12, MMA &/or holoTC; amniotic fluid B12 C: Women with unaffected pregnancies O: Presence of NTDs Studies of cohort or case-control design	17 case-control studies; mean sample sizes 33 cases & 93 controls	"Five studies estimated the risk of NTDs in relation to low B12, MMA or holoTC. These were "*significantly increased in three studies, with a non-significant trend in the fourth There seems to be a moderate association between low maternal B12 status and the risk of foetal NTDs*."

Author year	Databases searched and search date	Inclusion criteria	Included studies	Conclusions
Sukumar 2016[125]	Medline/PubMed, Embase, Global Health, Commonwealth Agricultural Bureau & CINAHL, Scopus December 2014	**Prevalence review**: P: Healthy pregnant women (aged 18–45 years) without major comorbidities, in any trimester including delivery I: B12 testing C: NA O: Worldwide prevalence of B12 deficiency **Low birth weight review**: P: Pregnancy outcomes / newborns I: Maternal testing in pregnancy of B12, MMA, holoTC, or Hcy – or testing of cord blood C: NA O: Prevalence of low birth weight	**Prevalence review:** 57 studies including (16 longitudinal (n = 34 results) and 41 cross-sectional) providing a total of 75 results **Low birth weight review**: 23 studies Observational studies English language	B12 insufficiency during pregnancy is common (25% women in included studies), particularly in the Indian subcontinent and Eastern Mediterranean, with B12 concentrations decreasing from the first to the third trimester. "*There is no consistent association between B12 insufficiency and low birth weight.*"
Wang 2012[126]	English language: PubMed, Medline, Elsevier Science & Springer Link Chinese languages: China National Knowledge Infrastructure, Vip information & China biology medical literature database July 2010	P: Women with a prior or current NTD-affected pregnancy I: Serum B12, MMA &/or holoTC C: Women with unaffected pregnancies O: Presence of NTDs	9 studies (567 cases & 1,566 controls) Cohort or case-control study designs English or Chinese languages	*"Low maternal B12 status could be an important risk factor for the development of fetal NTDs."*

Abbreviations

B12 = vitamin B12, **CI** = confidence interval, **CINAHL** = Cumulative Index to Nursing and Allied Health Literature, **Hcy** = homocysteine, **holoTC** = holotranscobalamin II, **MMA** = methylmalonic acid, **NA** = not applicable, **NTD** = neural tube defect, **OR** = odds ratio, **PICO** = population, intervention, comparator and outcome, **pmol/L** = picomoles per litre.

12.2 Evidence tables for linked evidence, primary research studies

Table 42 Characteristics of primary studies evaluating the diagnostic accuracy

Author; country; clinical trial ID	Population; sample size; study design	Population characteristics	Sensitivity/specificity	Vitamin B12 deficiency threshold	Conclusions
Campos 2020[33] Mexico and Switzerland	Mixed patient population, 11,833, retrospective paired two gate diagnostic test accuracy study Samples from consecutive clinical samples obtained between December 2006 and October 2018 with isolated or simultaneous measurements of HoloTC, MMA, Hcy.	Baseline characteristics of the 9,464 patients: Age (median years): 54 [36,68] Female (n): 5616 (59%) **Patient B12/biomarker levels (95% CI)** holoTC (pmol/L): 64 (45-93) B12 (pmol/L): 286 (217-381) MMA(nmol/L):177 (137-237) Hcy (umol/L):12.2 (9.7-15.3) 4cB12: -elevated B12 (4cB12>1.5): 0.89% -B12 adequate (4cB12 -0.5 to 1.5): 89.7% Low B12 (4cB12 -1.5 to -0.51): 8.9% Possible B12 deficiency (4cB12 -1.51 to -2.5): 0.5% Probable B12 deficiency (4cB12 <-2.5):0.06%	Sensitivity/specificity Optimum decision point (sensitivity/specificity): *Subclinical B12 deficiency (4cB12<-0.5 and >-1.5)* • HoloTC Sensitivity: 85.7% Specificity: 81.2% • Vitamin B12 Sensitivity 86.1% Specificity 77.7% • MMA Sensitivity 81.8% Specificity 83.4% *Possible or probably B12 deficiency (4cB12<-1.5)* • HoloTC Sensitivity 93.1% Specificity 96.0% • Vitamin B12 Sensitivity 94.8% Specificity 92.3% • MMA Sensitivity 94.8% Specificity 96.4%	Optimum decision point: *Subclinical B12 deficiency (4cB12<-0.5 and >-1.5)* • HoloTC <45 pmol/L • B12 <229 pmol/L • MMA >0.245 µmol/L *Possible or probably B12 deficiency (4cB12<-1.5)* • HoloTC <27 pmol/L • B12 <167 pmol/L • MMA >0.466 µmol/L	• For women 50 years and older, holoTC seems to be the preferred first-line marker for the detection of subclinical B12 deficiency
Heil 2012[113] the Netherlands	Mixed patients population with suspected vitamin B12 deficiency (November 2006-July 2007); 360 patients selected by the centres;	Age (mean years): 59.0 (19-100) Male (%): 38 Prevalence of metabolic B12 deficiency (MMA>0.45umol/L): 13%	Sensitivity/specificity • Vitamin B12 <145 pmol/L Sensitivity: 53% Specificity: 81%	Normal holoTC: 21-117 pmol/L Normal vitamin B12: 145-637 pmol/L	• An absolute improvement of 10–20% in sensitivity can be reached by replacing vitamin B12

Author; country; clinical trial ID	Population; sample size; study design	Population characteristics	Sensitivity/specificity	Vitamin B12 deficiency threshold	Conclusions
	retrospective paired two gate diagnostic test accuracy study	Decreased vitamin B12: 24% Decreased holoTC: 18% Abnormal creatinine males: 0% Abnormal creatinine females: 0.9%	• Vitamin B12 <180 pmol/L Sensitivity: 64% Specificity: 64% • HoloTC <21 pmol/L Sensitivity: 64% Specificity: 88% • HoloTC <32 pmol/L Sensitivity: 83% Specificity: 60%	Normal MMA: 0.09-0.45 µmol/L *Creatinine measured however doesn't appear to have influenced sample inclusion in study. Optimal Cut off HoloTC: 19-36 pmol/L Optimal Cut off Vitamin B12 180 pmol/L (lower cut-off resulted in sensitivity <50%, higher cut-off resulted in specificity <50%. Grey range of 136-216 pmol/L	by holoTC in screening for metabolic vitamin B12 deficiency depending on the chosen cut-off value • HoloTC at 32 pmol/L (compared to 21 pmol/L) increased specificity from 64% to 83%. Specificity decreased accordingly from 88% to 60% Increasing the (B12) cut off value from 145 pmol/L to 180 pmol/L, increased sensitivity from 53% to 64% and decreased specificity from 81% to 64%. Recommendations: • all patients with holoTC <32 pmol/L are treated with cobalamin • MMA is measured as a secondary test to confirm diagnosis in all patients with

Author; country; clinical trial ID	Population; sample size; study design	Population characteristics	Sensitivity/specificity	Vitamin B12 deficiency threshold	Conclusions
					holoTC <32 pmol/L • treatment is started in patients with holoTC <21 pmol/L • MMA (>0.45 µmol/L) is measured in patients whose holoTC is between 21 and 32 pmol/L to confirm diagnosis. • HoloTC has a better diagnostic accuracy than vitamin B12 and can replace the existing vitamin B12 assay as a primary screening test in patients suspected of vitamin B12 deficiency. • A cut-off value of 32 pmol/L can be considered in screening for metabolic vitamin B12 deficiency (defined by MMA 0.45 µmol/L) in a mixed patient population.
Herrmann, 2013[114]	Samples sent for total vitamin B12 assay; 1,359 serum samples; prospective paired	Patients with normal serum creatinine: 1,034	Sensitivity/specificity • Vitamin B12	Creatinine: ≤97.2 µmol/L MMA: >0.3 µmol/L	• The diagnostic power of holoTC seems insufficient to

Author; country; clinical trial ID	Population; sample size; study design	Population characteristics	Sensitivity/specificity	Vitamin B12 deficiency threshold	Conclusions
Germany	two gate diagnostic test accuracy study Samples were anonymous with no clinical information.	Patients with elevated serum creatinine: 325 Elevated MMA (>300 nmol/L) in patients with normal serum creatinine: 18.6% Elevated MMA (>300 nmol/L) in patients with elevated serum creatinine: 77.8% HoloTC range (pmol/L): 5-546 Total vitamin B12 range (pmol/L): 9-849	Sensitivity: 72% Specificity: 41% • HoloTC Sensitivity: 72% Specificity: 54%	HoloTC: <22 pmol/L (optimal <35 pmol/L) Vitamin B12: <227 pmol/L	distinguish deficient from nondeficient individuals with high reliability. • normal holoTC is common in individuals with elevated MMA, especially those with a decline in renal function. • Implementing a grey range of holoTC (between 90% sensitivity and 90% specificity) was suggested Recommendation: • A grey range of holoTC 23-75 pmol/L • Patients with normal renal function with holoTC in the grey zone should be followed by MMA testing
Schrempf, 2011[115] Germany	Neurological vitamin B12 deficiency syndromes; 1,279 participants Retrospective paired two gate diagnostic test accuracy study	Reasons for analysis (n): Suspected peripheral neuropathy: 925 Cognitive disorder:107 Subacute combined degeneration: 76 Multiple sclerosis: 55	Sensitivity/specificity Reference: MMA>0.298 µmol/L • Vitamin B12: Sensitivity: 66.2%, Specificity: 62.1%	Normal B12:155-672 pmol/L HoloTC: <42 pmol/L MMA: >0.298 µmol/L	• The positive predictive values for holoTC and vitamin B12 were low and both were associated

Author; country; clinical trial ID	Population; sample size; study design	Population characteristics	Sensitivity/specificity	Vitamin B12 deficiency threshold	Conclusions
		Restless leg syndrome:38 Movement disorders:38 Motor neuron diseases:34 Various:6	• HoloTC: 56.3% sensitivity, 50.5% specificity		with more false-positive than true-positive test results.
Schwarz, 2015[116] Germany	From a natural medicine-oriented doctor's office, 77 samples[A] paired two gate diagnostic test accuracy study	Male (%): 49.4 Age (mean years): 55.09 Diet (n/N, %) Vegan: 16/77, 20.8% Vegetarian: 28/77, 38.4% Omnivorous: 33/77, 42.9% Median vitamin B12-CLIA (ng/L): 333 (246.5-442) Median vitamin B12-MTP (ng/L): 428.4 (332-540.2) Median holoTC (pmol/L):59 (40.3-84.5) Median MMA (nmol/L): 196 (138-271.5) Median Hcy (umol/L): 11.1 (8.5-14.2)	Sensitivity/specificity HoloTC cut-off level at 35 pmol/L* (probable deficiency): • B12 CLIA: <155 pmol sensitivity: 53% specificity: 93% • B12 MTP: <212 pmol/L sensitivity: 71% Specificity: 95% • MMA >0.30 µmol/L Sensitivity: 41% Specificity: 90% HoloTC cut-off level at 50 pmol/L (possible/subclinical deficiency): • B12 CLIA: 155 pmol sensitivity (38%), specificity (94%) • B12 MTP: 212 pmol/L sensitivity (50%), specificity (96%) • MMA >0.30 µmol/L sensitivity (40%), specificity (94%) Optimised from ROC: • B12 CLIA = 224 pmol/L sensitivity: 94% specificity: 80% • B12 MTP = 271 pmol/L Sensitivity: 94%, Specificity: 78%	HoloTC: <35 pmol/L* HoloTC: <50 pmol/L B12 CLIA: <155 pmol/L* B12 MTP: <212 pmol/L (obtained from regression curve with CLIA) MMA: >0.3 µmol/L	• Increasing the total B12 thresholds improves the predictive power <224 pmol/L for B12-CLIA and <272 pmol/L for B12-MTP • HoloTC has a broad grey area of uncertainty and MMA should only be applied as a confirmatory test.
Valente, 2011[117]	Elderly individuals, 700	Age (mean, range): 81, 63-97	Sensitivity and specificity	HoloTC <20 pmol/L	• Renal dysfunction

Author; country; clinical trial ID	Population; sample size; study design	Population characteristics	Sensitivity/specificity	Vitamin B12 deficiency threshold	Conclusions
UK and Ireland	participants, recruited as part of an ongoing observational cohort study (December 2008 to October 2009), paired two gate diagnostic test accuracy study	Control age (median, range): 31, 18-62 Women (%): 70 Mean red cell cobalamin (n, pmol/L): 700, 64 Mean holoTC (n, pmol/L): 699, 47 Mean serum B12 (n, pmol/L): 700, 254 Mean MMA (n, µmol/L): 700, 0.347 Mean Hcy (n, µmol/L):700, 16.3 Mean creatinine (n, µmol/L):699, 89 Taking multivitamin or B vitamins: 8% [controls] 6% [elderly] Low red cell cobalamin (%): 9.6 Low holoTC (%): 8.1 Low total B12 (%): 8.0 Abnormal MMA (%):41.7 Abnormal total Hcy (%): 52.2	• HoloTC sensitivity: 55 (CI 43-67) specificity 96 (CI 94-97) • Serum vitamin B12: Sensitivity: 33 (CI 22-45) Specificity 95 (CI 93-96) • MMA Sensitivity: 81 (69-89) Specificity: 63 (59-66)	sB12 <123 pmol/L MMA >0.36 µmol/L Reference test: Red cell cobalamin: <33 pmol/L	compromised the diagnostic accuracy of MMA but not holoTC or serum cobalamin. • holoTC may be a first-line diagnostic test vitamin B12 status.

Abbreviations

B12 = vitamin B12, **CI** = confidence interval, **CLIA** = chemiluminescent immunoassay, **Hcy** = homocysteine, **holoTC** = holotranscobalamin II, **MMA** = methylmalonic acid, **MTP** = microbiological test with microtitre plates, **s** = serum, **p** = plasma, **pmol/L** = picomoles per litre, **PPV** = positive predictive value, **ROC** = receiver operating characteristic curve, **µmol/L** = micromoles per litre.

Notes

* based on laboratory recommendation.

[A]: Excluded patients based on creatinine values/kidney function

Table 43 Summary of results: change in management

Study	Population, sample size	Design; follow-up	Vitamin B12 deficiency criteria	Conclusion
Hvas 2000 [59]	Patients presenting with increased P-MMA between 1995-1996 at three Danish district hospitals n = 177 patients	Cross-sectional, NA	P-MMA: 0.05-0.37 µmol/L until 30 June 1996 and 0.08-0.28 µmol/L thereafter P-B12: <200 pmol/L	A high MMA test result did not greatly affect the decision to treat or not treat.
Hvas 2002 [60]	Patients with increased P-MMA between 1997-1999 presenting to 10 Danish GPs in Odder Municipality, Aarhus County. n = 181 patients	Cross-sectional, NA	p-MMA: >0.28 µmol/L (included patients) compared with >0.44 µmol/L (Danish recommendation)	Generally recommendations were followed – a high MMA result (mild or severe) led to treatment.
Carmel 1986 [58]	Patients with low serum vitamin B12 levels in 1983-1984 at the Los Angeles County-University of Southern California Medical Center n = 250 patients	Cross-sectional, NA	Serum B12: <184 pmol/L	Only 34% of patients received adequate management based on serum vitamin B12 test results.

Abbreviations
B12 = vitamin B12, **GP** = general practitioner, **MMA** = methylmalonic acid, **NA** = not applicable, **p** = plasma, **pmol/L** = picomoles per litre, **µmol/L** = micromoles per litre.

Table 44 Effectiveness of change in management: Natural course of B12 deficiency (primary studies)

Author; country	Indication; comparisons; sample size	Design; follow-up	Vitamin B12 deficiency criteria	Findings
Andres 2006[142] France	Elderly patients with vitamin B12 deficiency; patients with pernicious anaemia vs food B12 malabsorption; n = 201	Cohort NR	s-B12: <148 pmol/L t-Hcy: >13 µmol/L	Patients with B12 deficiency reported neurologic, psychologic, and hematologic abnormalities.
Ars 2019[132] the Netherlands	Pregnant women and their children from the Generation R study; patients with normal folate/B12/Hcy vs low folate/B12/Hcy; n = 256	Case-control NR	p-B12: <150 pmol/L t-Hcy: >9.1 µmol/L	No association found between prenatal p-B12 and childhood emotional and behavioural problems, cognition or brain volume.
Bennett 2001[146] Israel	Vitamin B12 deficient patients with a history of recurrent fetal loss; NA; n = 14	Case series NR	Subnormal B12: <132 pmol/L	Patients with B12 deficiency had recurrent foetal loss followed by periods of infertility. Most patients had successful pregnancy following supplementation.

Author; country	Indication; comparisons; sample size	Design; follow-up	Vitamin B12 deficiency criteria	Findings
Bernard 1998[74] USA	Older patients (>65 years); patients with normal B12 vs B12 deficient; n = 303	Cross sectional NR	'Strict' B12 deficiency: <148 pmol/L 'Broad' B12 deficiency: <221 pmol/L with MMA or Hcy elevated by more than 2 standard deviations	Patients with strict B12 deficiency had increased pain and lower cognitive performance compared to patients with normal B12 levels.
Biancheri 2001[127] USA	Patients with early onset vitamin B12 deficiency; NA; n = 14	Case series NR	NR	Patients with B12 deficiency had low birth weight, feeding difficulties, developmental delays failure to thrive, hypotonia and haematological abnormalities. Limited improvements post-treatment. Long-term symptoms included hypotonia, nystagmus, strabismus, seizures, mental retardation, hyperactivity and facial abnormalities.
Biemans 2015[133] the Netherlands	Patients with type 2 diabetes using metformin; Patients with sufficient B12 vs deficient in B12 and patients with sufficient holoTC vs deficient in holoTC; n = 550	Case-control NR	B12: <148 pmol/L HoloTC: <21 pmol/L	B12 deficiency associated with increased risk of depression and lower cognitive performance. No association between holoTC deficiency, depression and cognitive performance.
Cerit 2017[148] Cyprus	Patients who underwent coronary bypass grafting surgery; patients with normal B12 vs deficient in B12; n = 127	Case-control	B12: <148 pmol/L	B12 deficiency associated with increased progression of coronary artery disease.
Clarke 2007[81] UK	Elderly patients enrolled in the Oxford Healthy Aging Project; NA; n = 2,741	Longitudinal case series 10 years	NR	Low holoTC levels and high MMA levels were correlated with increased cognitive decline in older adults.
Cox-Klazinga, 1980[134] The Netherlands	Pernicious anaemia patients; patients receiving hydroxocobalamin vs no treatment; n = 40	Cohort study NR	NR	Patients with B12 deficiency reported neurological and haematological abnormalities.
Field 1995[145] UK	Patients referred to oral medicine clinic; NA; n = 14	Case series NR	s-B12: <125 pmol/L	Patients with B12 deficiency reported sore tongue, recurrent oral ulceration, and burning mouth.
Gadoth 2006[147] Israel	Older adults; patients with B12 deficiency vs patients who are not deficient in B12; n = 325	Case-control 6-9 months	B12: ≤147 pmol/L p-MMA: ≥0.24 µmol/L	Patients with B12 deficiency had decreased vibratory sensation, sensory neuropathy, and a history of confused states.

Author; country	Indication; comparisons; sample size	Design; follow-up	Vitamin B12 deficiency criteria	Findings
Garcia 2004[137] Canada	Older adults; patients with normal B12 vs low B12; n = 281	Cross sectional NR	Normal B12: 165-740 pmol/L, MCA: 0.6-0.2 µmol/L, Hcy: 5.1-13.9 nmol/L, MMA: 0.073-0.27 µmol/L	MCA and Hcy levels negatively correlated with cognitive performance.
Hall 1965[128] USA	Patients with pernicious anaemia; NA; n = 40	Case series NR	NR	Patients most commonly presented with dyspnoea, weakness, weight loss, and anorexia.
Healton 1991[129] USA	Patients with low vitamin B12 levels; NA; n = 143	Case series 37.6 months	s-B12: <150 pmol/L	Neurological abnormalities (e.g. paraesthesia) were the most frequently encountered symptom in patients with B12 deficiency.
Honzik 2010[149] Czech Republic	Infants breastfed from women with vitamin B12 deficiency; patients with profound B12 deficiency vs mild B12 deficiency; n = 40	Cohort NR	Normal B12: 150-664 pmol/L	Common symptoms reported in infants breastfed from mothers with B12 deficiency were failure to thrive, hypotonia, developmental delay, microcephaly and anaemia.
Hvas 2001[140] Denmark	Adults with p-MMA >0.28 µmol/L; NA; n = 432	Case series 1-3.9 years	p-MMA: >0.28 µmol/L p-Hcy: 5.8-11.9 µmol/L p-B12: 200-600 µmol/L	Patients with deficiency commonly reported neurological, haematological and gastrointestinal abnormalities. No association between p-MMA levels and neurological symptoms.
Kekki 1983[150] Finland	Patients with pernicious anaemia; Patients with pernicious anaemia vs computer-matched controls; n = 537	Case-control NR	NA	Dynamics of antral and body gastritis altered in patients with pernicious anaemia.
Krikke 2016[135] the Netherlands	Pregnant women and their children enrolled in the ABCD study; NA; n = 1,950	Case series 5-6 years	B12: <148 pmol/L	Low maternal B12 levels were associated with higher heart rates in the children.
Lindenbaum 1988[130] USA	Patients with low B12 levels; NA; n = 40	Case series 7 years	s-B12: <148 pmol/L	Patients with B12 deficiency reported neurological and haematological abnormalities.
Loukili 2004[143] France	Patients with B12 deficiency; NA; n = 49	Case series 2.5 years (mean)	B12: <148 pmol/L	Patients with B12 deficiency reported neurological and haematological abnormalities. One third of patients were asymptomatic

Author; country	Indication; comparisons; sample size	Design; follow-up	Vitamin B12 deficiency criteria	Findings
Meins 2000[151] Germany	Patients with probable Alzheimer's disease; Patients with subnormal B12 levels vs normal B12 levels; n = 73	Case-control NR	B12: <148 pmol/L	Low B12 levels were associated with irritability and disturbed behaviour in the context of dementia
Moelby 1997[97] Denmark	Patients with borderline B12 deficiency grey zone); patients with B12 levels between 99-119 pmol/L vs 120-145 pmol/L vs 146-178 pmol/L; n = 48	Cohort study 2 years	Reference intervals p-B12: <150-600 pmol/L p-B12 in patients >80 years:95-550 pmol/L p-MMA 0.05-0.37 µmol/L	Half of the patients with low B12 levels had neurological or psychiatric abnormalities. However, most were not deficient in B12.
Molloy 2009[152] Ireland	Women who were pregnant; patients with a history of pregnancies affected by NTD vs non-affected; n = 1,179	Case-control NR	B12 deficiency: <110 pmol/L Marginal B12 deficiency: 110–147 pmol/L	Low maternal B12 is associated with an increased risk of developing NTD during pregnancy.
Penninx 2000[131] USA	Older women with a physical disability; patients with no depression vs mild depression vs severe depression; n = 700	Case-control NR	B12: <148 pmol/L MMA: >0.27 µmol/L	Patients with severe depression were more likely to have B12 deficiency compared to non-depressed patients.
Ray 2007[138] Canada	Pregnant women; patients with a pregnancy affected by NTD vs non-affected; n = 511	Case-control study NR	s-holoTC: <55.3 pmol/L	Low holoTC levels were associated with an increased risk of NTD.
Reznikoff-Etievant 2002[144] France	Pregnant women; patients who had an early recurrent abortion or very early recurrent abortion vs healthy premenopausal women; n = 110	Case-control study NR	NR	Of women with low B12 and early abortions, 87.5% had very early recurrent abortions. Following supplementation 40% delivered a health child.
Roos 1977[141] Denmark	Patients with B12 deficiency post gastric surgery; patients with polyneuropathy vs slight peripheral involvement vs signs of myelopathy vs normal neurological investigation; n = 38	Case-control study NR	B12: ≤148 pmol/L	Half of the patients with B12 deficiency had peripheral neuropathy or polyneuropathy.
van Oijen 2007[136] the Netherlands	Patients with cardiovascular disease; NA; n = 211	Case series 5 years	s-B12: <250 pmol/L p-Hcy: >16 µmol/l	Increased p-Hcy level associated with increased risk of cardiovascular events and mortality. No association between B12 levels and cardiovascular events and mortality.

Author; country	Indication; comparisons; sample size	Design; follow-up	Vitamin B12 deficiency criteria	Findings
Wahlin 2001[153] Sweden	Older adults; patients with normal B12 and normal folate vs low B12, normal folate vs normal B12, low folate vs low B12 and low folate; n = 230	Case-control NR	B12: <200 pmol/L	There was no effect of B12 status on cognitive performance.
Wu 2013[139] Canada	Pregnant women and non-pregnant women with children; patients who are pregnant (16 and 32 weeks vs) not pregnant; n = 352	Case-control From 16 weeks of gestation to 9 months after birth	B12 deficiency: <148 pmol/L Marginal B12: 148-220 pmol/L	Maternal B12 deficiency associated with slower growth rate in boys (but not girls) compared to non-deficient mothers.

Abbreviations
B12 = vitamin B12, **FCM** = food-cobalamin malabsorption, **Hcy** = homocysteine, **HoloTC** = holotranscobalamin II, **MCA** = methylcitric acid, **MMA** = methylmalonic acid, **NTD** = neural tube defects, **NA** = not applicable, **NR** = not reported, **p** = plasma, **pmol/L** = picomoles per litre, **s** = serum, **t** = total, **UK** = United Kingdom, **USA** = United States of America, **µmol/L** = micromoles per litre.
Notes
[a] Reported in manuscript as 180 to 800 pg/L (i.e. 0.13-0.59 pmol/L), however this cut off would be inconsistent with current literature. It has been assumed at an error and corrected to pg/mL.

12.3 Test thresholds of diagnostic accuracy studies

Table 45 Test thresholds of studies included

Study	Threshold
Primary studies published after the Willis review	
Campos 2020[33]	Total vitamin B12: <167 pmol/L HoloTC: <27 pmol/L MMA: >0.47 µmol/L
Heil 2012[113]	Total vitamin B12: <145 pmol/L HoloTC: <21 pmol/L MMA: >0.45 µmol/L
Herrmann 2013[114]	Total vitamin B12: N/A HoloTC: <22 pmol/L MMA: >0.3 µmol/L
Schrempf 2011[115]	Total vitamin B12: <156 pmol/L HoloTC: <19 pmol/L MMA: >0.47 µmol/L
Schwarz 2015[116]	Total vitamin B12 (CLIA): <155 pmol/L Total vitamin B12 (MTP): <212 pmol/L HoloTC: <35 (35–50 grey zone) pmol/L MMA: >0.3µmol/L
Valente 2011[117]	Total vitamin B12: <123 pmol/L HoloTC: <20 pmol/L MMA: >0.36 µmol/L

Study	Threshold
Primary studies included in the Willis review	
Andres 2005[73]	s-B12: <148 pmol/L
Bernard 1998[74]	S-B12: s-B12 <148 pmol/L Grey zone B12: 148 <s-B12 pmol/L< 221 MMA and Hcy threshold: elevated by more than two standard deviations above mean
Bolann 2000[75]	s-B12 thresholds: <116 pmol/L and serum B12: <150 pmol/L s-MMA: >0.26 µmol/L (the upper reference limit) with at least 50% reduction after cobalamin supplementation.
Brett 1994[76]	s-B12: <148 pmol/L
Carmel 1992[77]	B12 deficiency deoxyuridine suppression: normal <8.5% B12: <185 pmol/L s-MMA >0.271 µmol/L
Carmel 1995[78]	Reference interval B12 in healthy volunteers 332 pmol/L (5-95% confidence limits) B12: <140 pmol/L MMA: > 0.376 µmol/L
Carmel 1996[79]	s-B12: <140 pmol/L s-MMA: >0.37 µmol/L
Carmel 1999[80]	reference ranges: s-B12: <140 pmol/L MMA (first 2 years): 0.079-0.376 µmol/L MMA (final year): 0.05-0.37 µmol/L
Clarke 2003[82]	B12: <150 pmol/L Grey zone B12: 150-200 pmol/L MMA: >0.35 µmol/L
Clarke 2007[81]	s-B12: <200 pmol/L holoTC: <45 pmol/L MMA: >0.45 or 0.75 µmol/L [total sample inc. patients with abnormal renal function]
Fora 2005[83]	suboptimal s-B12: <164 pmol/L
Goringe 2006[84]	B12: <125 pmol/L holoTC: <38 pmol/L MMA: >0.47 µmol/L
Green 2005[85]	Total B12: <148 pmol/L holoTC: <35 pmol/L
Herrrman 2003[86]	s-B12: <156 pmol/L or <265 pmol/L holoTC: <35 pmol/L MMA: >0.376 µmol/L (3 SD above healthy controls)
Holleland 1999[87]	s-B12: <170 pmol/L s-MMA: 0.05-0.26 µmol/L Diagnosing functional B12 deficiency s-MMA: >0.376 µmol/L
Hvas 2003[88]	HoloTC: <50 pmol/L MMA (possibly deficient): >0.29 µmol/L and >0.40 µmol/L MMA (likely deficient): >0.70 µmol/L p-B12: <200 pmol/L
Hvas 2005[89]	p-holoTC: <37 pmol/L MMA: >0.75 µmol/L, and ≥ 0.28 µmol/L

Study	Threshold
	p-B12: <200 pmol/L
Joosten 1993[61]	s-B12: <125 pmol/L s-MMA: >0.28 µmol/L but used 0.053-0.376 µmol/L
Kwok 2004[90]	MMA/creatinine: >4.8 µmol/L s-MMA: >0.4 µmol/L elevated >0.3 µmol/L mildly elevated subnormal: s-B12 <150 pmol/L and <300 pmol/L
Leaver 2004[91]	B12: <198 pmol/L MMA: >0.35 µmol/L holoTC: <27 pmol/L
Lindenbaum 1990[92]	Based on mean ± 3 SD) Normal s-MMA 0.053-0.376 µmol/L s-B12: <200 pmol/L
Lindenbaum 1994[62]	MMA: >0.376 µmol/L (normal range 0.053-0.376 µmol/L) s-B12: cut-off 258 pmol/L (normal range 148-665 pmol/L)
Lindgren 1997[94]	s-B12: <200 pmol/L Hospital definedreference s-B12: <130 pmol/L s-MMA: >0.4 µmol/L
Lindgren 1999[93]	s-B12 <200 pmol/L reference intervals (hospital defined normal levels) p-B12: 130-740 pmol/L (1st method) 200-600 pmol/L p-holoTC: <35 pmol/L s-MMA: upper reference limit 0.4 µmol/L
Lloyd-Wright 2003[95]	reference intervals (pre-set) holoTC: <50 pmol/L MMA: >0.28 µmol/L B12: ≥132 pmol/L Reference (as determined by study) holoTC: <25 pmol/L (likely deficient) B12: <88.5 pmol/L MMA >0.75 µmol/L
Matchar 1994[63]	s-B12 deficiency <147 pmol/L cut off s-B12 133 pmol/L (lower limit of normal)
Metz 1995[65]	B12 metabolic deficiency MMA: > 0.376 µmol/L (i.e. 3 SD above the control mean) s-B12: <148 pmol/L MMA: >0.375 µmol/L control s-MMA: 0.059-0.318 µmol/L
Metz1996[64]	s-B12: <150 pmol/L HoloTC: <30 pmol/L
Miller 2006[96]	B-12: <148 pmol/L holoTC: <35 pmol/L MMA: >0.35 µmol/L
Moelby 1990[98]	s-B12 deficiency: <100 pmol/L Normal ranges: B12: 135-585 pmol/L

Study	Threshold
	s-MMA: 0.05-0.34 µmol/L
Moelby 1997[97]	Reference ranges p-B12: 150-600 pmol/L 95-550 pmol/L(if age >80 years) p-MMA: 0.05-0.37 µmol/L
Naurath 1995[99]	Reference ranges s-B12: 103-406 pmol/L s-MMA: 0.062-0.247 µmol/L
Nilsson 1994[67]	s-B12: <150 pmol/L
Nilsson 1997[66]	s-B12: <150 pmol/L Upper reference limit [95th percentile] p-MMA: 0.41 µmol/L
Nilsson 1999[68]	Reference intervals s-B12: 150-650 pmol/L p-MMA: <0.41 µmol/L
Nilsson 2004[69]	Reference limits p-MMA: <0.41 µmol/L s-B12: 150-650 pmol/L
Norman 1993[100]	s-B12 boil-charcoal separation assay: 133-664 pmol/L [grey zone: 74-133] s-B12 no boil-solid phase separation procedure: 148-664 pmol/L low-normal s-B12: 133-258 pmol/L s-MMA: 0.073-0.271 µmol/L Deficiency s-MMA: >0.380 µmol/L
Obeid 2007[101]	B12: <156 pmol/L MMA: >0.299 µmol/L HoloTC: <36 pmol/L
Pennypacker 1992[70]	s-B12: <221 pmol/L s-MMA and/or Hcy: >3 SD Normal ranges s-B12: 148-590 pmol/L s-MMA: ± 3 SD 0.043-0.376 µmol/L ± 2 SD 0.073-0.271 µmol/L
Petchkrua 2003[102]	Subnormal s-B12 <162 pmol/L Supranormal MMA >0.279 µmol/L
Pflipsen 2009[103]	B12 deficiency B12: <74 pmol/L MMA: >0.243 µmol/L Normal ranges B-12: >258 pmol/L MMA: 0.088-0.243 µmol/L
Ray 2000[104]	Low s-B12: <120 pmol/L Intermediate B12: 120-150 pmol/L
Refsum 2001[105]	B12 deficiency t-B12: <150 pmol/L s-MMA: >0.26 µmol/L

Study	Threshold
	holoTC: <35 pmol/L
Saperstein 2003[106]	B12: <116 pmol/L s-MMA: >0.4 µmol/L
Savage 1994[107]	Normal MMA: 0.053-0.376 µmol/L s-B12 deficiency: <148 pmol/L
Selhub 1999[108]	Low B12: <185 pmol/L
Solomon 2005[37]	B12: <148 pmol/L Normal s-MMA: 0.09-0.25 µmol/L (first 4 years), 0.09-0.279 µmol/L (last 6 years) (> 0.376 µmol/L considered elevated)
Stabler 1990[109]	Normal s-B12: >147 pmol/L B12 deficiency S-B12: <118 pmol/L s-MMA and Hcy: >3 SD above normal
Sumner 1996[24]	B12 deficiency s-B12: <221 pmol/L s-MMA: >0.271 µmol/L
Ubbink 1993[71]	Normal ranges p-B12: 200-700 pmol/L
Van Asselt 1998[110]	B12 deficiency B12: <260 pmol/L p-MMA: >0.32 µmol/L
Vrethem 2003[111]	Reference values B12: 140-540 pmol/L P-MMA: <0.37 µmol/L
Wickramasinghe 1993[72]	s-B12: 122-505 pmol/L holoTC: 13-244 ng/L
Wickramasinghe 1996[112]	holoTC: 12.9-544.7 ng/L

Abbreviations

B12 = vitamin B12, **CLIA** = chemiluminescent immunoassay, **Hcy** = homocysteine, **holoTC** = holotranscobalamin II, **MTP** = microbiological tests with microtitre plates, **MMA** = methylmalonic acid, **NA** = not applicabe, **ng/L** = nanograms per litre, **s** = serum, **p** = plasma, **pmol/L** = picomoles per litre, **SD** = standard deviations, **TC** = transcobalamin, **µmol/L** = micromoles per litre.

Appendix C: Quality appraisal of the included trials

Table 46 Diagnostic accuracy, change in management and effectiveness of change in management: quality of systematic reviews and HTAs (AMSTAR)

CRITERION	Accuracy	Change in management	Effectiveness of change in management											
	Willis 2011[34]	Hvas 2003 (HTA)[191]	Andres 2018[118]	Butler 2006*[119]	Chan 2016†[19]	Mahawar 2018[120]	Moore 2012[32]	Petridou 2016[123]	Ray 2003[124]	Smelt 2017[122]	Smelt 2018[121]	Sukumar 2016[125]	Wang 2012[126]	Wang 2018[8]
1. Was an '*a priori*' design provided?	Y	N	P	Y	N	N	P	N	N	P	N	P	P	Y
2. Was there duplicate study selection and data extraction?	Y	N	P	Y	N	N	N	Y	N	P	P	Y	N	Y
3. Was a comprehensive literature search performed?	Y	Y	Y	Y	N	N	P	N	N	Y	Y	Y	Y	Y
4. Was grey literature eligible for inclusion?	Y	N	Y	Y	N	N	N	N	N	N	N	N	N	Y
5. Was a list of studies (included and excluded) provided?	Y	P	P	Y	P	P	N	P	P	P	P	P	P	Y
6. Were the characteristics of the included studies provided?	Y	Y	Y	Y	Y	Y	P	Y	Y	Y	Y	Y	P	Y
7. Was the scientific quality of the included studies assessed and documented?	Y	N	N	Y	N	N	N	Y	N	Y	Y	Y	N	Y
8. Was the scientific quality of the included studies used appropriately in formulating conclusions?	Y	N	N	Y	N	N	N	Y	N	Y	Y	Y	P	Y
9. Were the methods used to combine the findings of studies appropriate?	Y	N/A	NA	NA	NA	NA	NA	Y	NA	NA	Y	Y	Y	NA
10. Was the likelihood of publication bias assessed?	N	N/A	NA	NA	NA	NA	NA	Y	NA	NA	N	Y	Y	NA
11. Was a conflict of interest statement included?	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y

Abbreviations

P = partially satisfied, **N** = no, **NA** = not applicable, **Y** = yes.

Notes

Partially satisfied criteria (P) was awarded 0.5 points.

* Butler 2006[119] was an abbreviated version of a Cochrane review by Vidal-Alaball.[194] The Cochrane review was also accessed for study detail.

† Not formally a systematic review.

Table 47 Diagnostic accuracy: Quality appraisal of primary studies (QUADAS)

Study	Risk of Bias				Applicability concerns		
	Patient selection	Index test(s)	Reference standard(s)	Flow and timing	Patient selection	Index test(s)	Reference standard(s)
Campos 2020[33]	?	😊	?	😊	😊	😊	?
Heil 2012[113]	☹	☹	?	😊	?	😊	?
Herrmann 2013[114]	?	😊	?	😊	?	☹	?
Schrempf 2011[115]	?	😊	?	😊	☹*	😊	?
Schwarz 2015[116]	☹	☹	?	😊	☹	😊	?
Valente 2011[117]	😊	😊	?	😊	☹**	😊	☹

Abbreviations

😊 = low risk, ☹ = high risk, ? = unclear risk.

Notes

* Subpopulation: representative of psychiatric patients, but not the broader population defined in the PICO.

** Population only included patients <60 with mild cognitive impairment.

Table 48 Change in management: quality of primary studies (IHE case series checklist)

IHE Domain	Carmel 1986[58]	Hvas 2000[59]	Hvas 2002[60]
Study objective			
1. Objective clearly stated	Y	Y	Y
Study design			
2. Prospective	Y	N	N
3. Multicentre	N	Y	Y
4. Consecutive recruitment	Y	Y	Y
Study population			
5. Were patient characteristics included?	Y	Y	P
6. Eligibility criteria clearly stated	Y	Y	Y
7. Did patient enter the study at a similar point in the disease	NA	NA	NA
Intervention and co-intervention			
8. Was the method of intervention clearly described? [a]	Y	Y	Y
9. Were additional interventions clearly described?	NA	NA	NA
Outcome measure			
10. Were relevant outcome measures established *a priori*?	?	?	?
11. Were outcome assessors blinded to the intervention?	N	N	N
12. Were the outcomes measured using appropriate objective methods?	Y	P	P
13. Were the relevant outcome measures made before and after the intervention?	NA	NA	NA
Statistical analysis			
14. Were the statistical tests used to assess the relevant outcomes appropriate?	Y	Y	Y
Results and conclusions			
15. Was follow-up long enough for important events and outcomes to occur?	Y	Y	Y
16. Were losses to follow-up reported?	NA	NA	NA
17. Did study provide estimates of random variability in the data analysis of relevant outcomes?	Y	Y	Y
18. Were the adverse events reported?	NA	NA	NA
19. Were the conclusions supported by results?	Y	Y	Y
Competing interest and sources of support			
20. Were both competing interests and sources of support for the study reported?	P	P	N

Abbreviations
IHE = Institute for Health Economics case series checklist, **N** = no, **NA** = not applicable, **P** = partial/uncertain, **Y** = yes.
Notes
a = question amended from IHE case series study quality appraisal tool.

Table 49 Natural course of disease studies: quality of the single-arm trials (IHE)

Quality domain	Bennett 2001[146]	Biancheri 2001[127]	Clarke 2007[81]	Field 1995[145]	Hall 1965[128]	Healton 1991[129]	Hvas 2001[140]	Krikke 2016[150]	Lindenbaum 1988[130]	Loukili 2004[143]	Van Oijen 2007[136]
Study objective											
Objective clearly stated	N	Y	Y	Y	Y	Y	Y	Y	N	Y	Y
Study design											
Prospective	N	N	U	N	N	N	Y	Y	U	N	U
Multicentre	N	U	Y	N	N	Y	N	Y	N	Y	N
Consecutive recruitment	U	U	U	U	Y	U	U	U	U	U	U
Study population											
Were patient characteristics included?	N	Y	Y	P	N	Y	P	Y	P	P	Y
Eligibility criteria clearly stated	N	P	Y	Y	P	Y	Y	Y	Y	Y	Y
Did patient enter the study at a similar point in the disease	U	N	N	Y	Y	N	U	U	N	N	N
Method of diagnosis and co-intervention											
Was the method of diagnosis clearly described? [a]	Y	Y	N	Y	Y	Y	N	Y	Y	Y	Y
Were additional interventions clearly described?	Y	Y	NA	NA	NA	NA	NA	NA	NA	NA	NA
Outcome measure											
Were relevant outcome measures established *a priori*?	U	Y	Y	Y	N	Y	Y	Y	Y	Y	Y
Were outcome assessors blinded?	N	N	U	N	U	U	U	U	U	U	U
Were the outcomes measured using appropriate objective methods?	Y	Y	Y	Y	NA	Y	Y	Y	P	Y	Y

Quality domain	Bennett 2001[146]	Biancheri 2001[127]	Clarke 2007[81]	Field 1995[145]	Hall 1965[128]	Healton 1991[129]	Hvas 2001[140]	Krikke 2016[150]	Lindenbaum 1988[130]	Loukili 2004[143]	Van Oijen 2007[136]
Were the relevant outcome measures made before and after the intervention?	Y	Y	Y	Y	NA	Y	Y	NA	P	NA	Y
Statistical analysis											
Were the statistical tests used to assess the relevant outcomes appropriate?	NA	NA	Y	NA	NA	Y	Y	Y	U	U	Y
Results and conclusions											
Was follow-up long enough for important events and outcomes to occur?	Y	Y	Y	Y	NA	Y	Y	Y	Y	N	Y
Were losses to follow-up reported?	U	N	Y	N	NA	Y	Y	Y	U	U	Y
Did study provide estimates of random variability in the data analysis of relevant outcomes?	N	N	Y	NA	NA	Y	Y	Y	N	Y	Y
Were the adverse events reported?	NA	NA	NA	NA	NA	Y	NA	NA	NA	NA	NA
Were the conclusions supported by results?	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Competing interest and sources of support											
Were both competing interests and sources of support for the study reported?	P	P	Y	P	N	N	Y	Y	N	N	N

Abbreviations

IHE = Institute for Health Economics case series checklist, **N** = no, **NA** =not applicable, **P** = partial, **U** = uncertain, **Y** = yes.

Notes

[a] = question amended from IHE case series study quality appraisal tool.

Table 50 Natural course of disease studies: quality of case-control trials (SIGN)

Quality domain	Ars 2019[132]	Bernard 1998[74]	Biemans 2015[133]	Cerit 2016[148]	Gadoth 2006[147]	Garcia 2004[137]	Kekki 1983[135]	Meins 2000[151]	Molloy 2009[152]	Pennix 2000[131]	Ray 2007[138]	Reznikoff-Etievant 2002[144]	Roos 1977[141]	Wahlin 2001[153]	Wu 2103[139]
Section 1. Internal validity															
1.1 The study addresses an appropriate and clearly focused question.	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Selection of subjects															
1.2 The cases and controls are taken from comparable populations	Y	Y	Y	Y	Y	Y	C	Y	Y	Y	Y	Y	Y	Y	Y
1.3 The same exclusion criteria are used for both cases and controls	Y	C	Y	Y	C	Y	C	Y	Y	Y	C	C	C	Y	C
1.4 What percentage of each group (cases and controls) participated in the study?	244/538 of control children eligible	303/303 a	550/992 b	126/127	Case, 113/1,167 Control, 212/1,167	NA	NA	73/73	Group 2 Case, 107/187 Control, 414/439	700/1,409a	Case, 89/89 Control, 422/434 c	NA	NA	230/528 a	NA
1.5 Comparison is made between participants and non-participants to establish their similarities or differences.	Y	Y	Y	Y	Y	N	N	Y	C	Y	Y	N	N	Y	N
1.6 Cases are clearly defined and differentiated from controls.	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y

Quality domain	Ars 2019[132]	Bernard 1998[74]	Biemans 2015[133]	Cerit 2016[148]	Gadoth 2006[147]	Garcia 2004[137]	Kekki 1983[135]	Meins 2000[151]	Molloy 2009[152]	Pennix 2000[131]	Ray 2007[138]	Reznikoff-Etievant 2002[144]	Roos 1977[141]	Wahlin 2001[153]	Wu 2103[139]
1.7 It is clearly established that controls are non-cases.	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Assessment															
1.8 Measures will have been taken to prevent knowledge of primary exposure influencing case ascertainment.	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
1.9 Exposure status is measured in a standard, valid and reliable way.	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	C	Y	Y	Y	Y
Confounding															
1.10 The main potential confounders are identified and taken into account in the design and analysis.	Y	C	Y	Y	N	Y	Y	Y	N	Y	Y	N	N	N	Y
Statistical analysis															
1.11 Confidence intervals are provided.	Y	Y	Y	Y	Y	Y	NA [c]	Y	Y	Y	Y	N	Y	Y	Y
Section 2. Overall assessment of the study															
2.1 How well was the study done to minimise the risk of bias or confounding?	Acceptable	Acceptable	Acceptable	Acceptable	Unacceptable	Acceptable	Acceptable	Acceptable	Unacceptable	Acceptable	Acceptable	Unacceptable	Unacceptable	Acceptable	Acceptable
2.2 Taking into account clinical considerations, your evaluation of the methodology used, and	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C

Quality domain	Ars 2019[132]	Bernard 1998[74]	Biemans 2015[133]	Cerit 2016[148]	Gadoth 2006[147]	Garcia 2004[137]	Kekki 1983[135]	Meins 2000[151]	Molloy 2009[152]	Pennix 2000[131]	Ray 2007[138]	Reznikoff-Etievant 2002[144]	Roos 1977[141]	Wahlin 2001[153]	Wu 2103[139]
the statistical power of the study, do you think there is clear evidence of an association between exposure and outcome?															
2.3 Are the results of this study directly applicable to the patient group targeted by this guideline?	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C

Abbreviations

C = can't say, **N** = no, **NA** = not applicable, **Y** = yes.

Notes

a = the number of eligible participants per study arm was not reported separately, **b** = registered for the study, **c** = study performed a mathematical model evaluating the stages of antral and body gastritis, uncertain if estimates of variance are appropriate.

Table 51 Natural course of disease studies: quality of cohort trials (SIGN)

Quality domain	Andres 2006[142]	Cox-Klazinga 1980[134]	Honzik 2010[149]	Moelby 1997[97]
Section 1. Internal validity				
1.1 The study addresses an appropriate and clearly focused question.	Y	Y	Y	Y
Selection of subjects				
1.2 The two groups being studied are selected from source populations that are comparable in all respects other than the factor under investigation.	Y	C	C	C
1.3 The study indicates how many of the people asked to take part did so, in each of the groups being studied.	Does not apply	Does not apply	Does not apply	Does not apply
1.4 The likelihood that some eligible subjects might have the outcome at the time of enrolment is assessed and taken into account in the analysis.	N	N	N	N
1.5 What percentage of individuals or clusters recruited into each arm of the study dropped out before the study was completed.	No dropouts	No dropouts	No dropouts	No dropouts
1.6 Comparison is made between full participants and those lost to follow up, by exposure status.	N	N	N	N
Assessment				
1.7 The outcomes are clearly defined.	Y	Y	Y	Y
1.8 The assessment of outcome is made blind to exposure status. If the study is retrospective this may not be applicable.	NA	NA	NA	NA
1.9 Where blinding was not possible, there is some recognition that knowledge of exposure status could have influenced the assessment of outcome.	N	N	N	N
1.10 The method of assessment of exposure is reliable.	Y	Y	Y	Y
1.11 Evidence from other sources is used to demonstrate that the method of outcome assessment is valid and reliable.	Y	Y	N	N

Quality domain	Andres 2006[142]	Cox-Klazinga 1980[134]	Honzik 2010[149]	Moelby 1997[97]
1.12 Exposure level or prognostic factor is assessed more than once.	Y	N	N	Y
Confounding				
1.13 The main potential confounders are identified and taken into account in the design and analysis.	N	N	N	N
Statistical analysis				
1.14 Have confidence intervals been provided?	N	N	Y*	Y
Section 2. Overall assessment of the study				
2.1 How well was the study done to minimise the risk of bias or confounding?	Unacceptable	Unacceptable	Unacceptable	Unacceptable
2.2 Taking into account clinical considerations, your evaluation of the methodology used, and the statistical power of the study, do you think there is clear evidence of an association between exposure and outcome?	C	C	C	C
2.3 Are the results of this study directly applicable to the patient group targeted in this guideline?	C	C	C	C

Abbreviations
C = can't say, **N** = no, **NA** = not applicable, **Y** = yes.
Notes
* = measures of variance provided.