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Health Technology Assessment (HTA)

HTA Short Report

Title	Folate testing
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Technology	Diagnostic folate testing (serum folate/red blood cell folate)
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Executive summary

Objective

The aim of this health technology assessment (HTA) is to evaluate the safety, effectiveness and economic impact of folate testing in an asymptomatic general population and in patients with suspected folate deficiency due to the presence of symptoms, underlying medical disorders or external factors. In addition, ethical, legal, social and organisational issues related to folate testing are investigated.

Methods

A systematic literature search was conducted on 3 databases (PubMed, Embase, Cochrane Library) up to 1 August 2022. In addition, grey literature sources were searched for clinical practice guidelines and ongoing clinical trials. Direct from-test-to-health-outcomes evidence was preferentially sought. This evidence refers to studies that compare groups of people receiving either the current diagnostic test or the proposed diagnostic test, and measures the differential impact of the tests on patient health outcomes. In the absence of this evidence a linked evidence approach was undertaken, including an evaluation of diagnostic accuracy, change in clinical management, and the impact of management decisions on clinical outcomes.

Risk of bias was assessed using appraisal tools appropriate to the included study designs: systematic reviews and meta-analyses were evaluated using the ASMTAR-2 tool, diagnostic accuracy studies were evaluated using the QUADAS-2 tool, single-arm studies were evaluated using the Institute of Health Economics (IHE) quality appraisal tool for case series. Due to the limited evidence available, meta-analysis was not possible. Therefore, the results are summarised narratively.

Clinical evaluation

No direct from-test-to-health-outcomes evidence was identified, thus a linked evidence approach was used to evaluate the safety and effectiveness of folate testing.

Diagnostic accuracy

A single study published in 1984, which measured red blood cell (RBC) folate in 110 patients compared to haematological and clinical findings as the reference standard reported the sensitivity of folate testing to be 96% and the specificity to be 71% depending on the group of patients measured. The diagnostic accuracy findings are based on very low certainty evidence, the single study informing these results was considered at high risk of bias, and there is a high risk the findings

are not applicable to Swiss practice given the test used is not reflective of currently methods; thus the results should be interpreted with great caution.

Change in management

No studies reported *change* in management (i.e. comparing treatment decisions made with and without folate test results); however, 8 single-arm studies reported clinician management decisions following a low folate test result. These studies had inherently high risk of bias due to their study design (either case series or case control). It was consistently reported that, among patients suspected of folate deficiency and warranting a test, rates of folate deficiency were low (0.4%–4.9%). Clinician response to folate test results was universally low across all studies; among patients with a low folate diagnosis, only 24–62% received supplementation. There are concerns about the applicability of this data to the Swiss context, as most were collected from countries with mandatory folic acid fortification; however, a single study from Denmark (without mandatory fortification) reported results that were consistent with the rest of the evidence. Overall, the certainty of the management outcome data was considered very low due to risk of bias and applicability concerns.

Impact of change in management

Due to the limited evidence for accuracy, and the absence of evidence for *change* in management, the impact of change in management could not be assessed.

Safety of folic acid supplementation without testing

An umbrella review of meta-analyses indicates that folic acid supplementation is generally considered safe. Supplementation was associated with a decreased risk of a range of chronic condition and adverse health outcomes; however, higher rates of prostate cancer (RR 1.24, 95% CI 1.04, 1.49) and colorectal adenoma (RR 1.34, 95% CI 1.06, 1.70) were reported. With the exception of colorectal adenoma, these results were informed by high- or moderate-quality meta-analyses of randomised controlled trials (RCTs) as assessed by GRADE. Data from observational studies also showed an association between maternal folic acid supplementation and subsequent child asthma and/or wheeze for some measurements of these outcomes; however, data was mixed and this association is considered uncertain.

Budget impact assessment

Based on the limited evidence for the effectiveness of folate testing, cost-effectiveness analysis was not conducted. A budget impact assessment was conducted, which projects the current trends in the utilisation of folate testing and folic acid supplementation from 2022–2026. Assuming no change to the current reimbursement policies, the total budget expenditure on folate testing is projected to

increase from CHF 14.3 million in 2022 to CHF 21.5 million in 2026; folic acid supplementation is projected to increase from CHF 7.9 million in 2022 to CHF 9.3 million in 2026. Due to the limited evidence for the effectiveness of folate testing, scenarios investigating the impact of possible policy changes were not modelled.

Ethical, legal, social and organisational (ELSO) issues

A total of 8 studies were identified that addressed various ethical, social and organisational aspects of folate testing. No studies reporting legal issues were identified. Most studies identified addressed organisational issues relating to test restriction. Unsurprisingly, the 4 studies that described interventions to restrict folate testing noted significant cost savings. Ethical issues identified included potential adverse events from taking a supplement, a potential scenario owing to false positive test results or incorrect diagnosis in the absence of a test. Studies on social issues were in relation to physicians' opinions and concerns regarding an initiative to reduce folate test orders, which were mostly supportive, and citizens' opinions on test disinvestment.

Guidelines and existing HTAs

Five clinical practice guidelines (CPGs) were identified that focused on 2 specific populations; patients undergoing bariatric surgery and patients with suspected neurological deficiency as indicated by worsening gait ataxia. None of the CPGs were on folate testing, specifically. Evidence informing recommendations was either not reported or was of low quality (i.e. expert opinion). Only one CPG reported a threshold for identifying a folate deficient patient, and none made recommendations regarding timing or frequency of testing or follow-up testing in the case of deficiency. The searches identified 4 health technology assessments (HTAs) on folate testing (3 Canadian, 1 Australian). The findings were generally congruent with this current HTA, that there is a lack of evidence available to determine the effectiveness of folate testing.

Conclusions

Due to an absence of evidence the safety and effectiveness of folate testing is uncertain. There is no direct evidence examining the impact of folate testing on patient outcomes; similarly, evidence of the diagnostic accuracy of folate tests is limited to a single study published in 1984 with a high risk of bias and questionable applicability to current practice. There are no studies examining change in management based on the results of folate testing compared to no folate testing. These results broadly reflect the same results of prior HTA reports published on the topic.

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Abbreviations and acronyms

AMSTAR 2	A Measurement Tool to Assess Systematic Reviews 2
CI	confidence interval
CPG	clinical practice guideline
DNA	Deoxyribonucleic acid
EAE	effectiveness, appropriateness and economic efficiency
ELSO	Ethical, legal, social, organisational
FOPH	Federal Office of Public health
GRADE	Grading of Recommendations, Assessment, Development and Evaluations
HTA	Health Technology Assessment
IHE	Institute of Health Economics
MCV	mean corpuscular volume
MTHFR	methylenetetrahydrofolate reductase
NA	not applicable
NHMRC	National Health and Medical Research Council
NR	not reported
PICO (EO)	population, intervention, comparator, outcome, (economic outcomes)
QUADAS-2	Quality Assessment of Diagnostic Accuracy Studies
RBC	red blood cell
RoB-2	Cochrane risk-of-bias tool, version 2
SD	standard deviation
USA	United States of America
WHO	World Health Organization

Objective of the HTA report

The objective of a health technology assessment (HTA) is to generate a focused assessment of various aspects of a health technology. The analytic methods applied to assess the value of using a health technology, their execution and the results are described. The analytical process is comparative, systematic and transparent, and involves multiple stakeholders. The domains covered in an HTA report include clinical effectiveness and safety, costs, cost-effectiveness and budget impact, and ethical, legal, social 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

Each HTA topic entails a policy and a research question. In healthcare, a policy question is a request to regulate a reimbursement policy and is aimed at securing financing of health technologies. Such a request, related to a particular health technology, typically addresses an existing controversy around a technology.

In Switzerland, there are currently no limitations on mandatory insurance coverage of folate tests.[1] A sharp increase in the number of folate tests has been observed, with the number of folate serum tests ordered increasing from 510,589 to 738,164 and the number of folate red blood cell (RBC) tests increasing from 57,827 to 96,445 between 2016 and 2020, with no corresponding change in medical necessity (***Table 1***).[2] Limitation of coverage (e.g. to specific indications) could be considered if the effectiveness, appropriateness and economic efficiency (EAE) criteria of folate testing are not fulfilled or in light of the increasing utilisation patterns.[1] Therefore, this HTA has been proposed to evaluate the EAE criteria of carrying out folate tests.

Table 1 Number of claims and costs of folate tests in Switzerland, 2016 to 2020

	Year				
	2016	**2017**	**2018**	**2019**	**2020**
Folate test, serum					
Total number of tests ordered	510,589	635,547	714,417	767,569	738,164
Sum reimbursed (CHF) by mandatory insurance	6,721,285	8,387,991	9,441,249	10,138,331	9,634,587
Folate test, RBC					
Total number of tests ordered	57,827	75,405	88,296	98,886	96,445
Sum reimbursed (CHF) by mandatory insurance	1,219,706	1,590,306	1,863,050	2,087,323	2,024,705

Abbreviations

CHF = Swiss francs; **RBC** = red blood cell.

Source

Tarifpool: SASIS AG[2]

2 Medical background

2.1 Function of folate

Folate is the natural form of vitamin B9, an essential, water-soluble vitamin required for the synthesis of nucleic acids.[3] Most folate is present in its inactive form (5-methyltetrahydrofolate (5-methyl THF) in blood serum. After entering the cells, 5-methyl THF demethylates to tetrahydrofolate (THF), which is the biologically active form of folate involved in folate-dependent enzymatic reactions. These reactions are important for synthesising nitrogenous bases in nucleic acids and also for the maturation of RBCs.

Vitamin B12 is a cofactor required for the demethylation of 5-methyl THFA. In the absence of Vitamin B12, folate remains in its inactive form.[4] As a result, cell division is impaired and toxic metabolites accumulate. Thus, a deficiency of either folate or vitamin B12 will impair the production of THFA. The impairment of DNA synthesis prevents cell division in the bone marrow, leading to the production of large RBCs (megaloblastic anaemia).[5] Vitamin B12 deficiency also results in elevated methylmalonic acid levels and neurological signs that are not seen with folate deficiency. For this reason, vitamin B12 status is often checked before administering folic acid supplementation due to a suspected folate deficiency anaemia.[3]

Homocysteine, an amino acid which serves as an intermediate in methionine metabolism, requires both folate and vitamin B12 for its metabolism.[6] As such, deficiencies in either folate or B12 may also result in increased levels of homocysteine (hyperhomocysteinemia).

2.2 Sources of folate

Folate is found naturally in a wide variety of foods including leafy green vegetables and animal products including eggs, seafood and cheese; however, approximately 50% of the folate naturally present in food is bioavailable.[7] Folic acid is the synthetic form of folate used in supplements and the fortification of foods.[3]

Given the need for adequate folate levels prior to conception to prevent neural tube defects, many health organisations recommend women of reproductive age take folic acid supplements. Some countries, including the United States of America (USA), Canada and Australia, have mandatory folic acid fortification of staple foods. Across European countries, voluntary and mandatory fortification policies vary.[8] In Switzerland, approximately 200 food products are enriched with folic acid by the food industry on a voluntary basis but there is no mandatory fortification of foods.[9]

2.3 Causes of folate deficiency

Adults need approximately 400 mcg of folate per day to replenish daily losses, with deficiency taking approximately 8 to 16 weeks to become evident.[10] Poor diet, owing to poverty, is a known cause of nutritional deficiency.[11] Folate deficiency due to decreased intake is also observed in old age,[12] in people who have undergone bariatric surgery,[13] alcoholism,[14] and in people on fad diets.[15] Other causes of folate deficiency can be classified into 3 categories: increased requirements, impaired absorption and genetic folate disorders.

Increased requirements

Increased requirements for folate, leading to deficiency, can occur due to pregnancy and lactation, and diseases such as haemolytic anaemia, leukaemia, and aggressive lymphomas (owing to increased cellular turnover). Exfoliative dermatitis and haemodialysis cause folate deficiency through increased loss.[5 11]

Impaired absorption

Folate deficiency as a function of impaired absorption is associated with:

- **Alcoholism** interferes with folate absorption and uptake, accelerates folate breakdown and increases its excretion by the kidneys.[14] People who are heavily alcohol dependent may also have a poor diet, resulting in inadequate nutrient intake.
- **Medical conditions** that affect nutrient absorption include tropical sprue, coeliac disease, short bowel syndrome and inflammatory bowel disease.[10]
- **Prescription medications** such as methotrexate, a drug used to treat a range of disorders including rheumatoid arthritis, psoriasis, asthma, inflammatory bowel disease and cancer, are folate antagonists. Anticonvulsants, such as phenytoin, used in the treatment of epilepsy, decrease serum as well as liver folate levels.[16 17]

Genetic folate disorders

Congenital folate disorders lead to folate deficiency through either the defective absorption and transport of folate through cells, or the defective utilisation of folate due to enzyme deficiencies.[18] Hereditary folate malabsorption is a folate deficiency disorder with impaired intestinal folate absorption and impaired folate transport into the central nervous system. Signs include macrocytic anaemia, recurrent infections, and neurological deficits.[19] In contrast, the enzyme methylenetetrahydrofolate reductase (MTHFR) is involved in the conversion of 5,10-methyleneTHF to 5-methyl THF, which is the primary form of folate in the blood.[20] Severe MTHFR deficiency, is a rare autosomal recessive disorder associated with developmental delay, mental retardation, seizures and motor abnormalities.[17]

2.4 Incidence/prevalence of folate deficiency

No studies were identified that estimate the prevalence of folate deficiency in the general Swiss population; however, a recent cohort study investigating RBC folate levels in Swiss women (171 women of reproductive age and 177 who were pregnant) reported that 19.9% of women of reproductive age and 2.8% of pregnant women were folate deficient (RBC folate concentration <340 nmol/L [<150 ng/ml]). Of the women who were pregnant, 83% were on supplements containing folate, compared with 11% of women of reproductive age.[9]

Another study investigated folate levels across three age groups (60–69, 70–79 and ≥ 80 years) in 1,143 healthy Swiss senior citizens. The study found that whilst total serum folate significantly decreased with increasing age, RBC folate did not; however, for both serum and RBC folate a significant difference between males and females was reported, with lower levels in males. The reference interval, combining all gender and age strata for serum folate was 8.2 (90% CI: 7.6–8.5) to 42.8 (90% CI: 41.4–43.8) nmol/L (3.6 (90% CI: 3.4–3.8) to 18.9 (95% CI: 18.3–19.3) ng/ml). For RBC folate the reference interval, combining all gender and age strata was 444 (90% CI: 408–480) to 1934 (90% CI: 1,869–2,066) nmol/L (195.9 (90% CI: 180.1–211.8) to 853.5 (95% CI: 824.8–911.7) ng/ml).[21] Ferraro (2017) noted that the adequacy of folate status in Europe appears to vary widely across countries, with some populations, such as Norway, Sweden, Denmark and the Netherlands appearing to have suboptimal intake.[8] This is based on national survey data reporting on the nutritional behaviour of populations in European countries.[22]

2.5 Diagnosis of folate deficiency

The diagnosis of folate deficiency is complex, with no single test serving as a diagnostic reference standard.[5] In the absence of a reference standard, several factors are used to establish a diagnosis.

Physicians must first consider patient history, including dietary and drug history and alcohol use, in addition to physical presentation.[10] Physical symptoms that may raise suspicion of deficiency include anorexia, cognitive impairment, depression and dementia.[10] Patients with folate-deficient anaemia have symptoms similar to other anaemias, such as fatigue and pallor. In addition, in cases of severe folate deficiency they may have glossitis, gastrointestinal discomfort and diarrhoea.[15] It should be noted that biochemical and clinical evidence of deficiency can be observed in the absence of any physical symptoms.[23]

Direct methods of determining if a patient is folate deficient include measurement of folate concentration in the blood, by either serum folate or RBC folate tests. Depending on the patient's physical presentation, history and serum folate or RBC folate test results, a range of other laboratory tests may be conducted.

For example, plasma homocysteine, which accumulates because of folate deficiency[11], may be tested if serum/plasma folate levels indicate a deficiency or are indeterminate but the patient has no clinical symptoms.[24] If the cause of anaemia is being investigated other tests might include measuring mean corpuscular volume (MCV) and peripheral blood smears. Patients with elevated MCV may have a megaloblastic disorder; the presence of megaloblastic changes usually implies either a vitamin B12 or folate deficiency. The presence of oval macrocytes in a peripheral blood smear is also suggestive of a megaloblastic disorder.[5] As the metabolic roles of folate and vitamin B12 are closely linked, and both can cause megaloblastic macrocytic anaemia, patients evaluated for folate deficiency as the cause of megaloblastic macrocytic anaemia, are also often evaluated for vitamin B12 deficiency.[10]

With respect to serum and RBC folate tests, the only guideline identified on the diagnosis and treatment of folate disorders published by the British Committee for Standards in Haematology, states that there is no clear consensus as to what cut-off value is used to define deficiency with respect to serum values. However, the committee notes that clinicians conventionally have used a serum folate level of <7 nmol/L (<3.1 ng/ml) as indicative of folate deficiency (Grade 1B level of evidence = strong recommendation (confidence that the benefits outweigh the harm), moderate level of evidence).[23]

A scientific opinion publication by the European Food and Safety Authority states that serum folate concentrations <6.8 nmol/L (<3.0 mg/ml) and RBC folate concentrations <317 nmol/L (<140 ng/ml) are cut-off values indicative of folate deficiency, noting that the cut-offs for deficiency were determined in adults.[25] However, they further note that for the assessment of folate status, multiple measurements of serum folate should be taken over a period of several weeks or a single measurement should be combined with other biomarkers of folate status.[25] This is because serum folate levels vary with recent food intake and acute dietary changes can result in low levels without any significant tissue deficiency.[26] Thus, a single measurement of serum folate is not informative of folate status as it reflects folate levels at the time of blood collection, i.e. in response to recent food intake or acute dietary changes.[25]

The World Health Organization (WHO) has published recommended folate thresholds for monitoring trends in folate status in populations and the impact of public health interventions. The values (applicable to all age groups) are based on the concentration below which homocysteine levels start to rise. The cut-off for serum folate level is <10 nmol/L (<4.4 ng/ml) and the cut-off for RBC folate is <340 nmol/L (<150 ng/ml). The WHO notes that measurements obtained by radioimmunoassy need adjustment to make them comparable with the microbiological assay.[27] It should be noted that these cut-off values are for the purpose of monitoring folate status in populations and the impact of interventions such as folic acid fortification, not for the clinical assessment of folate deficiency.

The serum and RBC folate levels that analytical laboratories in Switzerland use as a cut-off value to diagnose folate deficiency are not published. Cut-off values for deficiency have been found to be dependent on the analytical platform used.[28]

2.6 Treatment of folate deficiency

Folate deficiency is treated with folic acid supplementation. Intravenous, subcutaneous or intramuscular formulations can be used for patients unable to take oral medications.[10] The amount necessary is dependent on the reason for the deficiency. Guidelines by the British Committee for Standards in Haematology recommend those outlined by the British National Formulary (Grade 1A level of evidence = strong recommendation [confidence that the benefits outweigh the harm], high quality of evidence). These are summarised in the guidelines as follows:

- *Folate deficient megaloblastic anaemia (due to dietary insufficiency, pregnancy or antiepileptics)*: 5 mg of folic acid daily for 4 months, except in pregnancy where it is continued until term, and up to 15 mg daily for 4 months in malabsorptive states.
- *Chronic haemolytic states and renal dialysis*: the prophylactic dose suggested is 5 mg daily to weekly, depending on the diet and rate of haemolysis.
- *Pregnancy*: the prophylactic dose suggested is 200 to 500 µg daily.[23]

The duration of therapy depends on whether cause of the initial deficiency continues. Patients with malabsorption disorders may require long-term treatment.[10]

3 Technology

3.1 Technology description

There are 2 types of laboratory tests funded in Switzerland by mandatory health insurance for the determination of a patient's folate status: serum folate tests and RBC (erythrocyte) folate tests. Serum folate is not strictly a diagnostic test for body folate depletion, as serum folate levels reflect recent dietary intake (short term status). Also, recent alcohol abuse may result in a low serum folate concentration without body depletion because alcohol interferes with the absorption of folate and speeds the rate that folate breaks down and is excreted from the body.[29] In comparison, RBC folate is an indicator of long term folate status, reflecting folate status during the past four months...[26] However, subnormal RBC folate levels may occur in patients with severe vitamin B12 deficiency and return to normal following vitamin B12 supplementation alone.[26] This is owing to an interrelationship between these two vitamins. Vitamin B12 is a cofactor required for the demethylation of 5-methyl THF (the inactive form of folate) to tetrahydrofolate (the biologically active form involved in folate dependent enzymatic reactions). Thus, a

deficiency in vitamin B12 leads to and increased proportion of folate trapped inside cells as 5-methyl THF.[10] The determination of folate in serum/plasma is reported to be the most common laboratory test used for folate status evaluation; however, there is disagreement among experts regarding the optimal folate assay to use.[28] Measurement of RBC folate is said to be more technically demanding, and thus more expensive, than serum folate measurement because of the greater pre-treatment it requires, which includes lysis of the RBCs and deconjugation of the polyglutamate folate forms present, and dilution of the intracellular folate down to a range that can be measured by the assay.[30 31] The pre-treatment involved in the analysis of RBC folate is reported to introduce several variables into the measurement process.[30] After measurement of the amount of folate present in the whole blood lysate, the RBC folate concentration is calculated using the patient's haematocrit. Measurement of haematocrit is said to introduce further uncertainty, and is another process unique to RBC folate determination.[30 31]

The British Committee for Standards in Haematology guideline on folate diagnosis states that RBC folate is not necessary because serum folate alone is sufficient in most cases (Grade 1A level of evidence = strong recommendation (confidence that the benefits outweigh the harm), high quality of evidence). However, in the presence of strong clinical suspicion of folate deficiency, despite a normal serum level, an RBC folate assay may be undertaken, having ruled out vitamin B12 deficiency (Grade 2B level of evidence = weak recommendation (magnitude of benefit is less certain), moderate quality of evidence).[23]

Several methods can be used in the assessment of folate in both serum and RBCs, including microbiological assays, folate-binding assays and high-performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS).[32] Microbiological assays were the original method used to measure both serum and RBC folate and are historically considered to be the reference standard measurement technique.[33] Automated immunoassays based on the competitive or non-competitive binding of labelled folate and endogenous folates to a folate-binding protein are now reported to be the most routinely used methods to determine serum and RBC folate in patients, owing to their lower cost and high throughput.[24 34 35] It is reported that competitive protein binding assaying is an expensive diagnostic tool and the laboratory costs are substantially equal for serum and RBC methods.[28]

Limitations of the automated folate binding assays have been reported, relating to the variable affinity the various forms of folate in the serum or RBCs have for the folate binding protein dependent on the assay conditions.[24] There are numerous automated platforms available for RBC and serum folate determination. A recently published comparison of 4 different immunoassays for serum folate determination found that the consistency of the results among these assays was not good and most method differences among the assays exceeded the acceptable quality specification.[35] Very significant differences in results have also been reported between commercial immunoassays for RBC folate.[34]

The methods used to analyse serum and RBC folate in Switzerland are not specified in the Analysenliste.[36]

3.2 Alternative technologies

Serum and RBC folate tests are the only tests reimbursed by mandatory health insurance in Switzerland for the specific evaluation of folate deficiency. It is noted that serum, blood or plasma homocysteine testing is listed on the Analysenliste (position number 1422.00, Tax point 27.0). Plasma homocysteine has been evaluated as a biomarker of folate status and has been observed to negatively correlate with serum folate and RBC folate in a number of studies.[30] However, it should be noted that it is not a specific indicator of folate status because deficiencies in other vitamins (vitamin B12, B6 and riboflavin) can also cause it to be elevated.[37] Folate guidelines published by the British Committee for Standards in Haematology recommend that plasma homocysteine should only be measured to confirm suspected folate deficiency in special circumstances.[23] It is not known whether homocysteine tests are being ordered by doctors in Switzerland for the evaluation of patients with suspected folate deficiency. The alternative to testing for folate is the initiation of folic acid supplementation based on the patient's risk factors and clinical symptoms.

3.3 Reimbursement status

In Switzerland, serum and RBC folate testing is reimbursed under mandatory health insurance through the Analysenliste (German), Liste des Analyses (French) or Elenco delle Analisi (Italian) (***Table 2***). There are no restrictions on either test.

Table 2 Tests available for the evaluation of folate deficiency in Switzerland (Analysenliste)

Designation	Limitations	Position number	Tax point
Folate, blood	None	1329.00	11.8
Folate, erythrocytes	None	1330.00	18.9

Notes
Analysenliste Edition: 1st August 2022
Source
Bundesamt für Gesundheit BAG 2022[36]

4 Research questions/Population, Intervention, Comparator, Outcomes (PICO)

In this section, the research questions, associated PICO criteria, eligible study designs and relevant languages are defined. The proposed questions addressing each component of the EAE criteria are described separately.[1]

4.1 Effectiveness outcomes: Direct from-test-to-health-outcomes evidence

For the evaluation of clinical outcomes, direct from-test-to-health-outcomes evidence was sought preferentially as it directly addresses the policy question (represented by arrow C in ***Figure 1***). Direct from-test-to-health-outcomes evidence refers to evidence from studies that measure the impact of conducting a test on a health outcome.[38] This includes studies in which the treatment decisions are directly linked to a test result; health outcomes are differentiated based on test results and subsequent treatment decisions.[38] No direct from-test-to-health-outcomes evidence was identified, necessitating the use of a linked evidence approach (represented by arrows D to F in ***Figure 1***).

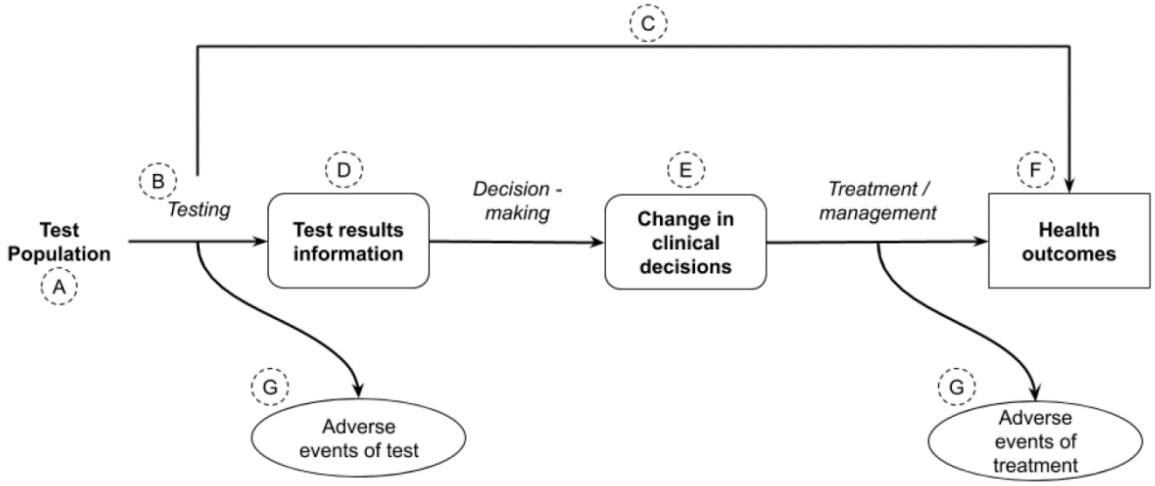

Figure 1 Evaluation framework for diagnostic tests

Source
Medical Services Advisory Committee[38]

4.2 Effectiveness outcomes: Linked evidence approach

A linked evidence approach was undertaken to evaluate the impact of folate testing on patient outcomes. In a linked evidence approach, the evidence generated by direct from-test-to-health-outcomes evidence is broken down into stages, each representing a different component of the pathway from test to health outcome.

The first step in the linked evidence approach is to investigate the clinical performance of the test (***Table 3***). Early phase studies establishing the analytical validity of the tests (test-retest reliability etc.) were not included. Subsequently, the impact of the test outcomes on patient management was evaluated (***Table 4***). The final step in the linked evidence approach was to investigate the impact of any potential changes in management on patient outcomes (***Table 5***).

Table 3 Research question and study selection criteria for linked evidence: diagnostic accuracy of folate testing

Research question	In the asymptomatic general population or in those with suspected folate deficiency due to the presence of symptoms, underlying medical disorders, or external factors, what is the diagnostic accuracy of folate testing?
Population*	1. Asymptomatic general population 2. Patients with suspected folate deficiency due to the presence of symptoms, underlying medical disorders, or external factors
Presentation	1. Symptoms of folate deficiency (see background section of protocol) 2. Increased requirement for folate (e.g. pregnancy) 3. Asymptomatic but with chronic disease linked to folate deficiency (see background section of protocol)
Prior tests	Not applicable
Index test(s)†	1. Diagnostic folate testing (serum folate test) 2. Diagnostic folate testing (red blood cell folate test)
Comparator test(s)	No test
Purpose	To quantify folic acid levels, in order to diagnose a symptomatic or asymptomatic folate deficiency (first-line diagnosis)
Outcome(s)	**Test accuracy**: sensitivity, specificity, false positives, false negatives, invalid/uninterpretable results, positive predictive value and negative predictive value (detection of subclinical deficiency). **Diagnostic yield**
Reference standard	There is no accepted reference test for folate deficiency. For the purposes of validating diagnostic accuracy results, any other test of folate (serum or red blood cell) or clinical monitoring of symptoms may be used (e.g. haematological or neurological symptoms).
Study design(s)	1. Systematic reviews and/or meta-analyses of diagnostic accuracy studies. 2. In the absence of systematic reviews for any of the index tests, primary diagnostic accuracy studies will be considered. 3. In the absence of diagnostic accuracy studies, diagnostic yield studies‡ will be considered.
Language(s)	English, French, German and Italian

Notes

* Data from each population will be presented separately unless reported in combination or if the population is not well defined; † Data from each test will be reported separately. The evaluation will also consider and report results where tests are conducted sequentially; ‡ Diagnostic yield studies evaluate the yield of diagnosed patients, as determined by an index test, without confirmation of the accuracy of this diagnosis by a reference standard.[39]

Table 4 Research question and study selection criteria for linked evidence: impact of folate testing on clinical management

Research question	In the asymptomatic general population or in those with suspected folate deficiency due to the presence of symptoms, underlying medical disorders or external factors, does folate testing prior to folic acid supplementation affect supplementation commencement, route and frequency compared to no testing?
Population*	1. Asymptomatic general population 2. Patients with suspected folate deficiency due to the presence of symptoms, underlying medical disorders, or external factors
Intervention†	1. Diagnostic folate testing (serum folate test) 2. Diagnostic folate testing (red blood cell folate test)
Comparator	No folate testing
Outcome(s)	% change in patients treated with supplementation % change in patients with a condition other than folate deficiency treated with unnecessary supplementation % change in the dosage, route or frequency of supplementation
Study design(s)	1. Systematic reviews and/or meta-analyses of RCTs or single arm trials 2. In the absence of systematic reviews and/or meta-analyses of RCTs or single arm trials, RCTs will be considered 3. In the absence of RCTs, single arm trials (i.e. diagnostic before-and-after studies, historical control studies) will be considered
Language(s)	English, French, German and Italian

Abbreviations
RCT = randomised controlled trial.
Notes
* Data from each population will be presented separately unless reported in combination or if the population is not well defined; † Data from each test will be reported separately. The evaluation will also consider and report results where tests are conducted sequentially.

Table 5 Research question and study selection criteria for linked evidence: impact of clinical management decisions on health outcomes

Research question(s)	1. Do changes in management have an impact on patients with folate deficiency (i.e. true positives)? 2. Do changes in management have an impact on patients suspected of folate deficiency who are incorrectly diagnosed (i.e. false positive and false negatives)?
Population*	1. Asymptomatic general population 2. Patients with suspected folate deficiency due to the presence of symptoms, underlying medical disorders, or external factors
Intervention	Folic acid supplementation
Comparators	No treatment
Outcomes†	1. Adverse events associated with folic acid supplementation in patients with folate deficiency (e.g. interactions with other medications, masking/ of vitamin B12 deficiency) 2. Adverse events associated with folic acid supplementation in patients with adequate folate levels (e.g. interactions with other medications, masking of vitamin B12 deficiency) 3. Resolution of symptoms associated with folate deficiency (anaemia, megaloblastic anaemia, leukopenia, thrombocytopenia and hyperhomocysteinemia) 4. Quality of life (improved, maintained or reduced) 5. Time to administration of appropriate method of supplementation 6. % change in number of patients taking prescribed supplements **Disease natural history**

	Natural progression of condition when untreated (morbidity)
Study design(s)	1. Systematic reviews and/or meta-analyses of RCTs or non-randomised studies. 2. In the absence of systematic reviews and/or meta-analyses, RCTs will be considered. 3. In the absence of RCTs, non-randomised studies will be considered.
Language(s)	English, French, German and Italian

Abbreviations
RCT = randomised controlled trial.
Notes
* Data from each population will be presented separately unless reported in combination or if the population is not well defined; † Outcomes will be reported at longest follow-up. Additionally, the list of symptoms associated with folate deficiency reported here is not exhaustive. All outcomes reported in the included literature will be considered, with prioritisation by a clinical expert if needed.

4.3 Safety outcomes

Table 6 Research question and study selection criteria for the safety of folate testing

Research question(s)	In the asymptomatic general population or in those with suspected folate deficiency due to the presence of symptoms, underlying medical disorders, or external factors, how safe is folic acid supplementation informed by testing compared to folic acid supplementation not informed by testing?
Population*	1. Asymptomatic general population 2. Patients with suspected folate deficiency due to the presence of symptoms, underlying medical disorders, or external factors
Intervention	Folic acid supplementation informed by a folate test
Comparators	Folic acid supplementation not informed by a folate test
Outcomes†	1. Adverse events associated with folate testing (i.e. venepuncture) 2. Adverse events associated with folic acid supplementation 3. Adverse events associated with untreated folate deficiency
Study design(s)	1. Systematic reviews and/or meta-analyses of RCTs or non-randomised studies 2. In the absence of systematic reviews and/or meta-analyses, RCTs will be considered 3. In the absence of RCTs, non-randomised studies will be considered
Language(s)	English, French, German and Italian

Abbreviations
RCT = randomised controlled trial.
Notes
* Data from each population will be presented separately unless reported in combination or if the population is not well defined; † Outcomes will be reported at longest follow-up. Additionally, the list of symptoms associated with folate deficiency reported here is not exhaustive. All outcomes reported in the included literature will be considered, with prioritisation by a clinical expert if needed.

4.4 Economic outcomes

Economic outcomes relate to the cost and cost-effectiveness or cost-utility of the medical service, ultimately reflecting the value for money offered by the service. These outcomes were planned to be evaluated by considering existing published literature where available, and through a *de novo* economic evaluation when existing literature was not available (***Table 7***). Due to the absence of clinical evidence on the effectiveness of folate testing on health outcomes, a *de novo* economic evaluation was not possible. A budgetary impact assessment was conducted.

Table 7 Research question and study selection criteria for economic outcomes

Research question(s)	1. What is the annual budgetary impact of folate testing and subsequent supplementation?
Population	1. Asymptomatic general population 2. Patients with suspected folate deficiency due to the presence of symptoms, underlying medical disorders, or external factors
Intervention	1. Diagnostic folate testing (serum folate test) 2. Diagnostic folate testing (red blood cell folate test)
Comparator	No testing
Outcomes	Direct medical costs (CHF): pharmaceutical costs; laboratory costs; outpatient and inpatient medical care costs. Quality-adjusted life years (e.g. calculated using SF-36, EQ-5D or equivalent metric) Incremental cost-effectiveness ratio or incremental cost utility ratio (i.e. cost per quality-adjusted life year)
Study design(s)	Cost-effectiveness/cost-utility analyses Budgetary impact analyses
Language(s)	English, French, German and Italian

Abbreviations
CHF = Swiss francs, **EQ-5D** = EuroQol five-dimensions questionnaire, **SF-36** = Short Form 36.

4.5 Appropriateness outcomes

The final domain in the EAE criteria deals with the appropriateness of the intervention. Issues related to ethical, legal, social or organisational matters relating to the use of folate testing were investigated in this domain, noting that only issues directly relevant to the policy question were described (***Table 8***).

Table 8 Research question and study selection criteria for appropriateness outcomes

Research question	Are there ethical, legal, social or organisational issues associated with folate testing and subsequent supplementation?
Population	Patients eligible for folate testing
Intervention	Supplementation following folate testing
Comparator	Supplementation with no testing
Outcomes*	**Ethical**: balance of benefits and harms (e.g. are there hidden or unintended consequences of conducting the test), autonomy (e.g. is the value of testing augmented by its impact on the autonomy of patients), equity (e.g. does the implementation of testing have impacts on equitable access to care across the population). **Legal**: can the limitation of this technology to certain populations pose ethical challenges which have not been considered in existing legislations and regulations. **Social**: patient preferences and expectations about folate testing, caregiver preferences and expectations about folate testing. **Organisational**: structure of the health system (e.g. are there standard cut-offs used to define folate deficiency), process-related costs (e.g. how would services be reoriented in the absence of folate testing and what costs would these incur), culture (e.g. how is folate testing accepted in practice).
Study design(s)	Systematic reviews, narrative reviews, randomised controlled trials, observational studies, cross-sectional studies (i.e. surveys), government reports.
Language(s)	English, French, German and Italian

Notes
* These outcomes have been defined in accordance with the EUnetHTA Core Model 3.0.[40]

5 Methodology: Safety and effectiveness outcomes

5.1 Literature search strategy

5.1.1 Databases and other sources

A systematic search of the scientific peer-reviewed literature addressing the research questions was performed in 3 databases (PubMed, Embase, Cochrane Library) on 26 July and 1 August 2022. No date restrictions were applied. The search strategies used are presented in ***Table 20*** to ***Table 23*** (***Appendix A***).

Searches were also conducted in ClinicalTrials.gov and the EU Clinical Trials Registry to identify ongoing clinical trials related to the research questions (***Table 24, Appendix A***).

Grey literature searches were conducted on HTA agency websites and international HTA databases

Table 27***, Appendix A***) using combinations of the keywords 'folate', 'folic acid', 'folacin', 'folate deficiency', 'folic acid deficiency', 'vitamin B9', 'vitamin B9 deficiency' and 'test*' to identify existing HTA reports on the same topic. Reference lists of included studies were pearled to identify additional studies.

5.1.2 Inclusion and exclusion criteria

Studies were selected for inclusion according to the research questions, PICO criteria, study designs and languages defined in ***Section 4***.

For the clinical questions, inclusion of existing systematic reviews (of moderate to high quality, per the A Measurement Tool to Assess Systematic Reviews 2 [AMSTAR 2] checklist) were preferentially sought. In the absence of eligible systematic reviews, primary studies that met the inclusion criteria were selected for inclusion, starting with the highest level of evidence relevant to each research question according to the National Health and Medical Research Council (NHMRC) hierarchy of evidence.[39]

Conference abstracts, letters to the editor and author responses, case reports and animal studies were excluded from the review.

5.1.3 Study selection

Two reviewers split the screening of all the titles and abstracts retrieved using the search strategies reported in ***Table 20*** to ***Table 23*** (***Appendix A***). Grey literature searches (for clinical trials and of CPG, specialty society and HTA websites) were carried out by a single reviewer. The full texts of articles deemed potentially relevant were reviewed independently by 2 reviewers. Any disagreements on article inclusions were resolved by consensus; if consensus could not be reached, a third independent reviewer was consulted to determine inclusion.

5.2 Data extraction, appraisal and synthesis

5.2.1 Data extraction

One reviewer 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. Data of interest included:

- study information: country, year, number of institutions, study design, inclusion/exclusion criteria, withdrawals, length of follow-up
- demographic information: number of participants, age, sex, comorbidities, indication for testing, prior testing
- intervention and comparator: type and method of intervention/comparator (including index test, reference test, dose and administration route), concomitant interventions
- outcomes of interest: diagnostic accuracy data, baseline, final or change from baseline scores in any of the predefined outcomes outlined in ***Section 4***
- any noteworthy features, limitations or differences in the study.

5.2.2 Critical appraisal

Two independent researchers conducted critical appraisal, with differences settled via consensus or by an independent reviewer where consensus could not be reached. Critical appraisal was conducted using different tools depending on the study design. Umbrella reviews of metanalyses were appraised using a modified AMSTAR 2 checklist;[41] diagnostic accuracy studies were appraised using the QUADAS-2 (Quality Assessment of Diagnostic Accuracy Studies Version 2) tool;[42] single-arm trials were appraised using the Institute of Health Economics (IHE) quality appraisal checklist for case series.[43]

The quality of evidence for each outcome was evaluated using the GRADE (Grading of Recommendation, Assessment, Development and Evaluations) approach.[44] The 5 domains of the GRADE framework (imprecision, inconsistency, indirectness, risk of bias and publication bias) were assessed according to the decision algorithm developed by Pollock (2016).[44 45] The overall strength of the evidence has been presented in 'Summary of Findings' tables generated in GRADEpro.[44]

5.2.3 Data synthesis

Data from each population have been presented separately, where possible. In addition, data for each type of folate test (i.e. serum folate test and RBC folate test) have been presented separately, where possible. Where available, results relating to the following subgroups have been discussed:

- Outcomes for subpopulations (e.g. patients planning to become pregnant, pregnant patients, elderly)

- Outcomes from studies conducted in settings with folate deficiency levels similar to those found in Switzerland
- Any other subgroup in the included studies, determined before the analysis of results, decided in conjunction with a clinical expert.

The approach to data synthesis for each research question depended on the available evidence. Given the type of evidence available, each linked evidence domain has been summarised narratively.

5.3 Changes from the research protocol

The original research protocol included safety outcomes as part of the linked evidence assessment of effectiveness. In the HTA report, a separate section was created for safety, as noted in ***Section 4***.

6 Clinical effectiveness and safety

6.1 Summary statement efficacy, effectiveness and safety

6.1.1 Direct evidence

No direct test-to-patient-outcome evidence on folate testing was identified.

6.1.2 Linked evidence

Diagnostic accuracy**:** One diagnostic cohort study measuring RBC folate in 110 patients reported the sensitivity of folate testing to be 96% and the specificity to 71%. The reference standard was confirmation of folate deficiency based on a range of haematological and clinical tests and dietary analysis. Not all patients had all tests. The diagnostic accuracy findings are based on very low certainty evidence. The single study informing these results was considered at high risk of bias, there is a high risk the findings are not applicable, and these results should be interpreted with caution.

Change in management**:** No studies reported *change* in management (i.e. comparing treatment decisions made with and without folate test results); however, 8 single-arm studies reported clinician management decisions following a low folate test result. These studies had inherently high risk of bias due to their study design. It was consistently reported that, among patients suspected of folate deficiency and warranting a test, rates of folate deficiency were low (0.4–4.9%). Clinician response to folate test results was universally low across all studies—among patients with a low folate diagnosis, 24–62% received supplementation. There are concerns about the applicability of these data as most were collected from countries with mandatory folic acid fortification; however, a single study from Denmark (without mandatory fortification) reported results consistent with the rest of the evidence. Folate deficiency levels reported by this study were 1.7% (of tests requested by hospital doctors) and 1.3% (of tests requested by general practitioners), whilst supplementation initiation when folate deficiency was detected was only 54%,

Impact from change in management**:** Due to the limited evidence for accuracy, and the absence of evidence for *change* in management, the impact of change in management could not be assessed.

Safety of folate testing and supplementation**:** An umbrella review of meta-analyses indicates that folic acid supplementation is generally considered safe. It was not reported whether any of the reviews included patients who were diagnosed as being deficient. Supplementation was associated with a decreased risk of a wide range of chronic diseases and adverse health outcomes but higher rates of prostate cancer (RR 1.24, 95% CI 1.04, 1.49) and colorectal adenoma (RR 1.34, 95% CI 1.06, 1.70).

Excluding colorectal adenoma, results were informed by high- or moderate-quality meta-analyses of randomised controlled trials (RCTs) as assessed by GRADE. Data from observational studies also showed an association between maternal folic acid supplementation and subsequent child asthma and/or wheeze for some measurements of these outcomes; however, data were mixed and this association is uncertain.

6.2 Search results

The results of the study selection process are presented in ***Figure 2***. The search results from each database are reported in ***Table 20*** to ***Table 23*** (***Appendix A***)***.*** After removal of duplicates, 6,653 records were screened by title/abstract. A total of 568 were reviewed by full text, of which 9 met the inclusion criteria. No eligible systematic reviews were identified; therefore, primary studies formed the basis of the clinical effectiveness and safety section. The list of studies excluded at full-text review, with reasons for exclusion, is available upon request.

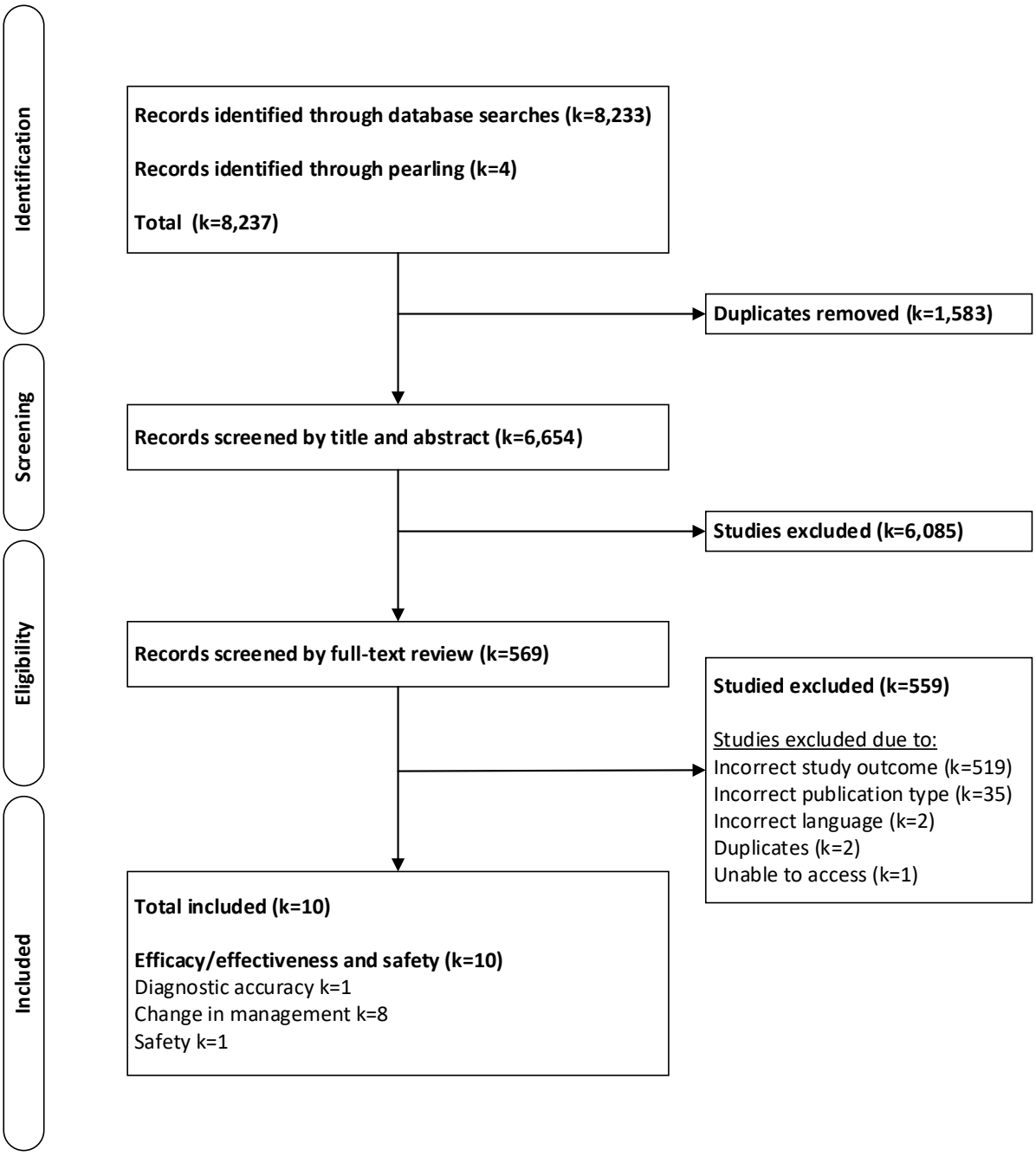

Figure 2 PRISMA flow chart

6.3 Direct from-test-to-health-outcomes evidence

No direct evidence was identified, therefore a linked evidence approach was taken.

6.4 Linked evidence: Diagnostic accuracy

6.4.1 Study characteristics

One diagnostic cohort study published in 1984 was identified that presented diagnostic accuracy data for RBC folate testing.[46] All folate samples sent for RBC folate testing at a tertiary hospital in the UK for

a 2.5-year period from January 1981 were assayed. Patients were assessed as being folate deficient based on a range of haematological and clinical tests including bone marrow aspirate, deoxyuridine suppression test, Schilling test, small bowel biopsy as well as dietary analysis. These tests were the reference standard against which the diagnostic accuracy of RBC test was assessed. Not all patients had all tests. Patients were enrolled in a single cohort, including all assays conducted during the period (indication for testing unspecified); a separate group of healthy controls was also included, but has not been included in this group is not eligible for folate testing in practice. The results of the tests were stratified into groups based on the test findings as follows: 1) definitely folate deficient (n = 18), 2) probably folate deficient (n=7), 3) definitely B12 deficient with no reason to suspect folate deficiency (n=35), 4) probably B12 deficient with no reason to suspect folate deficiency (n=3), 5) macrocytic but not folate deficient (n = 45), and 6) possibly deficient of both B12 and folic acid (i.e. equivocal results, n=2). The authors did not report how many patients referred for a folate test were included in the analysis. A threshold of 200 mcg/L was used to determine folate deficiency. This was derived from the lower bound of the 95% confidence interval in 200 heathy volunteers. The study is summarised in ***Table 28***, ***Appendix B***.

6.4.2 Risk of bias

The diagnostic accuracy study was appraised using the QUADAS- 2 tool[42] (***Table 31, Appendix C***). The study was judged as having an overall high risk of bias because not all patients received the same reference standard. Domain 1 (patient selection) was assessed as having an uncertain risk of bias, owing to the lack of description around the tested cohort. Domain 2 (index test) was assessed as having a low risk of bias. Domain 3 (reference standard) was assessed has having an uncertain risk of bias, as it is unclear how likely it is that the reference standard can correctly classify the target condition. Risk of bias in domain 4 (flow and timing) was assessed as high due to a failure to report the total number of eligible folate tests received during the period of the study and how many patients were excluded from the analysis. The study also failed to report sufficient details to assess the appropriateness and timing of the reference standard (clinical examination of patients).

There are strong concerns with the applicability of the study. Expert advice suggests the method used for folate level determination in the study (i.e. folic acid radioassay) is not representative of modern practice. It is also not clear whether the threshold development method and level determined are appropriate and applicable to modern practice. It is not known whether the indications for folate testing in this study are applicable to current usage, as this was not reported.

6.4.3 Results

Diagnostic accuracy data were available from a single study.[46] It is not clear if the study employed a one-gate (i.e. using a single set of selection criteria) or multiple-gate (i.e. using multiple sets of selection criteria) design due to inadequate reporting. Bain (1984) reported RBC folate testing had a sensitivity of 96% in patients with known or probable folate deficiency.[46] Specificity was 71% in patients who had a definite or probably B12 deficiency but no reason to suspect a folate deficiency, or macrocyclic haematology but no folate deficiency.[46] These results should be interpreted with caution. A clinical expert reported that the folic acid radioassay is no longer used (personal comment; haematologist). Thus, the results from this study are unlikely to be representative of the accuracy of folate testing occurring in Switzerland today. Serum results are not reported.

6.5 Linked evidence: Change in management

6.5.1 Study characteristics

No comparative evidence on change in management was identified. In total, 8 studies investigating management decisions following folate testing were identified and these are summarised in ***Table 29***, ***Appendix B***. All the studies were either case series in design, or case control studies; however, relevant data were only extracted from a single arm of the study.[47-54]

In total, 6 of the studies were conducted in the USA[47 49-53], one study was conducted in Denmark[48] and one in Israel[54]. All the studies retrospectively reviewed results from a consecutive series of folate tests conducted over a specified time period (4 months to 3 years). Regarding test interpretation, 5 studies measured serum folate using a threshold of 1.5–3 ng/ml (3.4–6.8 nmol/L) to indicate deficiency.[47 50-53] An additional study used a serum folate threshold of 5.6 nmol/L (2.5 ng/ml) to indicate deficiency.[54] Of these studies, 3 measured RBC folate; 2 used a threshold of 175 ng/ml (397 nmol/L)[49 50] while one used a threshold of 155 ng/ml (351 nmol/L).[48]

Three of the studies also defined a 'borderline or insufficient' folate status and used a range of 3.4–5.5 ng/ml (7.7–12.5 nmol/L),[52] 3.0–3.9 ng/ml (6.8–8.8 nmol/L)[53] or 3.0–4.0 ng/ml (6.8–9.1 nmol/L)[47] to describe this status.

6.5.2 Risk of bias

No change-in-management studies were identified, only case series studies reporting how folate testing informed management decisions were included. These studies are considered at inherently high risk of bias due to the study design. The quality of the 8 case series studies was appraised using the IHE Quality Appraisal Checklist (***Table 32***, ***Appendix C***).

All studies clearly stated objectives, used consecutive recruitment and measured relevant outcomes using objective methods. Further, all studies established relevant outcome *a priori* and clearly stated the eligibility criteria. All studies reported appropriate conclusions supported by the results. Four studies used a multicentre design.[48 51 52 54]

The major limitation of the evidence base was the retrospective study design used by all studies. Whilst the folate test results are probably at low risk of bias given this is an objective measure (i.e. a recorded value), the risk of bias regarding the management decision is uncertain. This is owing to the subjectivity and variability that may have occurred with patient chart recording, given it did not follow a predefined protocol.

All studies reported some relevant details of the folate test, including the type of folate measurement (RBC or serum) and the threshold used to determine a deficient result, receiving at least a partial score for this criterion. Three studies also reported details of the specific test used (including manufacturer).[49 50 53]

Competing interests and sources of funding were fully declared by Vinker and Ashaf[47 54], partially declared by Sign and Theisen[52 53] and not reported by Jaffe (1991), Robinson and Mladenovic (2001), Latif (2004) and Bor (2008).[48-51]

Follow-up time was unclear for all studies.

Eight criteria (listed below) were considered not applicable, due to the type of study design and the type of outcome measures reported:

- Did patient enter the study at a similar point in the disease?
- Were additional interventions clearly described?
- Were outcome assessors blinded to the intervention?
- Were the relevant outcome measures made before and after the intervention?
- Were the statistical tests used to assess the relevant outcomes appropriate?
- Were losses to follow-up reported?
- Did study provide estimates of random variability in the data analysis of relevant outcomes?
- Were the adverse events reported?

6.5.3 Results

No studies reported change in management; data were only available on management decisions following a folate test.

All studies reporting management post-folate testing were highly consistent in study methodology and findings.[47-54]

The detection rates of folate deficiency were low across all studies, ranging from 0.4–4.9%.[47-54] Of patients identified with folate deficiency, the mean rate of subsequent management with supplementation was 46.4% (268/577 patients across all studies, range 24%–62%).[47-54] Only Latif (2004) and Bor (2008) reported supplementation rates above 50%, with rates of 62% and 54%, respectively.[48 50]

Of these studies, 3 reported overall management decisions based on folate testing. Vinker (2013) calculated 1.9% of folate tests resulted in clinician response, Latif (2004) reported 0.9% of patients who were clinically suspected of folate deficiency received supplementation, and Robinson and Mladenovic reported the overall clinical response to folate testing was 0.5%.[50 51 54] Robinson and Mladenovic reported only 53% of folate results were recorded and only 24% were attributed to a possible cause.[51] Similarly, Bor (2008) reported that only 46% of low folate results were recorded in patient charts.[48]

Results from Bor (2008) are considered the most applicable to the Swiss context.[48] The study was conducted in Denmark, which, similar to Switzerland, does not have mandatory folic acid fortification. Results from this study were consistent with those from other studies, with low rates of folate deficiency detected (1.7% of tests requested by hospital doctors and 1.3% of tests requested by general practitioners) and low rates of supplement initiation when folate deficiency was identified (54%).

6.6 Linked evidence: Impact of change in management

Due to the absence of evidence on the diagnostic accuracy of folate testing and change in management, the impact of change in management was not investigated.

6.7 Safety of folate testing

No studies were identified that investigated the safety of folate testing. The testing procedure involves a venepuncture for blood draw, which is a common procedure, therefore it is not expected that there are substantial safety issues involved in the test itself.

6.8 Safety of folic acid supplementation

6.8.1 Study characteristics

Under both the intervention and comparator management strategies being investigated for this report, patients would be exposed to folic acid supplementation if folate deficiency was suspected or confirmed after testing. Therefore, the safety of supplementation is a key issue to consider.

One umbrella review of meta-analyses was identified that examined the association of folate with health outcomes.[55] The review pooled results from 154 meta-analyses of RCTs and 133 meta-analyses of

observational studies published in the Medline, Embase or Cochrane library databases up to May 2018. The quality of each included meta-analysis was appraised using AMSTAR 2. The studies were grouped by unique health comes and a risk ratio with 95% confidence interval was generated for each. Results from meta-analyses of RCTs and observational studies were reported separately. Where a risk ratio was statistically significant ($p < 0.05$) a prediction interval and between study heterogeneity was calculated. The GRADE (Grading of Recommendation, Assessment, Development and Evaluation) working group classification was used to assess the quality of the findings.

6.8.2 Risk of bias

The umbrella review by Bo (2020) was appraised using a modified version of the AMSTAR 2 assessment tool.[41] No validated appraisal tool was identified for appraising umbrella reviews; however, the framework provided by AMSTAR 2 is largely applicable to these types of review and was considered an appropriate choice, as the methodology for study identification, selection and extraction are the same as a for a traditional systematic review. Similarly, consideration of the quality appraisal, statistical methods and heterogeneity are important for umbrella reviews, albeit requiring a different methodology for these aspects.

The primary limitation of the review, and a source of bias, is that it excluded systematic reviews without meta-analyses and thus the findings may not reflect the entire evidence base. In addition, it is not clear whether there was overlap of studies included in reviews on the same outcomes, which would result in a misleading, overly precise estimate.[56] The review used GRADE to assess the quality of evidence for each outcome; however, this tool was designed for primary studies, not systematic review evidence. It is reported that there is currently no guidance on appropriate use and interpretation of the GRADE tool when assessing the quality of the evidence base on systematic reviews.[56] Other imitations of the review by Bo (2020) were the failure to establish that the review was guided by a protocol and a failure to publish and justify the list of excluded studies. The included systematic reviews' assessment of publication bias was noted; however, this was not discussed or analysed further. Full results of the appraisal are presented in ***Table 33***, ***Appendix C***.

6.8.3 Results

An umbrella review of meta-analyses on the association between folate and health outcomes indicated that folic acid supplementation is generally considered to be safe. Synthesis of meta-analyses deemed to be of high or moderate GRADE classification by the authors showed that folic acid supplementation was associated with decreased risks of: elective termination of pregnancy for foetal anomalies, megaloblastic anaemia, neural tube defects, cardiovascular disease (among people with pre-existing diseases, liver toxicity (in patients receiving methotrexate), gestational hypertension/preeclampsia, low

predelivery serum folate, decreased scores on the Hamilton Depression Rating Scale and levels of plasma homocysteine (both among patients with type 2 diabetes and the general population) increased levels of birth weight, red blood cell folate, and serum/plasma folate. However, folic acid supplementation was associated with higher rates of prostate cancer in patients with pre-existing diseases (RR 1.24, 95% CI 1.04, 1.49) and colorectal adenoma in the general population (RR 1.34, 95% CI 1.06, 1.70). [46] The increased risk of colorectal adenoma was in patients who had been on folic acid supplementation for over three years and this evidence was of serious risk of bias as assessed by AMSTAR 2 and very low quality as assessed by GRADE. For prostate cancer, the result was driven by results from a single study, removal of which resulted in non-significant results. However, it was noted that the study was of low risk of bias and there is no rationale to justify its removal from the analysis. The authors hypothesised that these inconsistent findings for prostate cancer may therefore represent heterogeneity in the included populations and study design.[46]

Pooled data from observational studies also showed an association between:

- periconceptional pregnancy supplementation and child asthma and wheezing (RR 1.05, 95% CI 1.02, 1.09)
- early pregnancy folic acid supplementation and child wheezing as an isolated symptom (RR 1.06, RR 1.02, 1.09).[55]

It should be noted that other measures of the association between maternal folic acid supplementation and infant respiratory symptoms, as listed below, did not reach levels of statistical significance:

- pregnancy supplementation and child asthma (RR 1.06, 95% CI 0.99, 1.14)
- pregnancy supplementation and wheeze (RR 1.05, 95% CI 0.95, 1.15)
- pregnancy supplementation other than early pregnancy and wheeze (RR 1.00, 95% CI 0.96, 1.03).

These results are therefore uncertain, based on meta-analyses of observational data, and should be interpreted with caution.

Isolated single cases of anaphylaxis due to folic acid supplementation have been reported.[57-59] However, this is considered extremely rare, with 13 cases described in the literature between 1949 and 2015.[58]

It has been theorised that increased folic acid intake may mask a vitamin B12 deficiency by correcting macrocytosis.[60-62] However, this is based on observational data[62] and results are inconsistent.[60] It is unknown whether this is merely a theoretical risk or to what extent increasing supplementation will increase the harms associated with B12 deficiency. A retrospective analysis of laboratory results in the USA reported that the proportion of patients with low B12 concentration but without anaemia did not

differ during the periods of pre- and post-folic acid fortification, suggesting no increase in masked B12 deficiency.[63]

6.9 GRADE summary of findings

The overall certainty findings of the key diagnostic accuracy and management outcomes are summarised in ***Table 9*** and ***Table 10***. The GRADE Working Group has defined the following grades of evidence:

- 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.[44]

Diagnostic accuracy outcomes were appraised as having 'very low' certainty. These findings were informed by a single study, at high risk of bias. Further, there were serious concerns about the applicability of the study because the methodology used to determine folate results is outdated and not currently used in clinical practice. There is also uncertainty in the prevalence of folate deficiency in the Swiss population. A single study of 348 women estimated this as being 19.9% in women of childbearing age and 2.8% in pregnant women.[9] At the time of collection 19/171 (11%) of the women of reproductive age and 146/177 (83%) of the pregnant women consumed supplements containing folate. It is unlikely that the results from this study can be reasonably extrapolated to the wider Swiss population that may undergo folate testing. It is questionable to use the prevalence data in combination with the diagnostic accuracy data, as the methodology for the folate test in the diagnostic accuracy study differs from that used in the prevalence study, and these were measured in different population groups. Therefore, while we have provided an evaluation of the diagnostic accuracy of folate testing in ***Table 9***, these results should be interpreted with a very high degree of caution.

A single management outcome (rate of supplementation) was appraised using GRADE methodology, as this outcome was reported consistently by all management studies and is the key outcome for this section. The certainty of these results was rated 'very low' due to concerns around study design and risk of bias and concerns around the applicability of the results measured in countries with mandatory supplementation being applied to Switzerland.

Table 9 GRADE Summary of findings table: diagnostic accuracy outcomes

Patient or population: patients at risk for or suspected of folate deficiency
Setting: hospital inpatient
Reference test: A range of clinical tests including bone marrow aspirate, deoxyuridine suppression test, Schilling test, dietary analysis, small bowel biopsy. Note: not all patients had all tests.
Estimated sensitivity: 0.96 | **Estimated specificity**: 0.71

Test result	Number of results per 1,000 patients tested (95% CI)		Number of participants (studies)	Certainty of the Evidence (GRADE)
	Prevalence 19.9% Typically seen in women of reproductive age in Switzerland	**Prevalence 2.8%** Typically seen in pregnant women in Switzerland (note: 83% on supplements containing folate)		
True positives	191	27	25 (1)	⨁◯◯◯ **Very low**[a,b]
False negatives	8	1		
True negatives	569	690	95 (1)	⨁◯◯◯ **Very low**[a,b]
False positives	232	282		

CI = confidence interval
Explanations: **a** =High risk of bias as assessed using the QUADAS-2 tool[42]; **b** = High concerns about the applicability of the test methodology and the threshold used compared to those used in Switzerland today.

Table 10 GRADE Summary of findings table: management outcomes

Patient or population: patients suspected of or at risk for folate deficiency
Setting: inpatient and outpatient folate tests
Intervention: folate testing
Comparison: none

Outcomes	Total supplemented/total with a low folate result	№ of participants (studies)	Certainty of the evidence (GRADE)
Folic acid supplementation rates	268/577 (46.4%)	577 (8 observational studies)	⊕◯◯◯ Very low[a,b]

Explanations: **a** = Case series design appraised using IHE tool; **b** = No comparative evidence on change in management, no evidence on the comparator

6.10 Ongoing clinical trials

A search for ongoing clinical trials for folate testing was undertaken on 6 September 2022 in ClinicalTrials.gov and the EU Clinical Trials Registry using a combination of the following terms: folate AND test OR testing OR diagnosis OR diagnostic. A total of 73 hits were retrieved from ClinicalTrials.gov and 29 hits from the EU Clinical Trials Registry. Of these, none were related to the accuracy of RBC or serum folate testing.

7 Clinical practice guideline review

Summary statement clinical practice guideline review

There is a paucity of evidence-based clinical practice guidelines (CPG) on folate testing published since 2017. Recently published guidelines focus primarily on the use of folate testing as part of the perioperative management of patients undergoing bariatric surgery; one other guideline recommended folate testing in patients with progressively worsening gait ataxia. Notably, only one of the guidelines proposed a threshold for interpreting the findings of the test, and the evidence supporting the recommendations were typically either very low (i.e. based on expert opinion), or not reported. One guideline from the Royal College of Pathologists (UK) recommends against repeat folate testing in patients with folate deficiency.

7.1 Methods

7.1.1 Search strategy

The systematic literature searches outlined in ***Section 5.1*** were used to identify CPGs related to the use of folate testing. Grey literature searches were also conducted on CPG websites (***Table 25***, ***Appendix A***) and specialty websites (***Table 26***, ***Appendix A***) using combinations of keywords relating to 'folate', 'folic acid', 'folacin', 'folate deficiency', 'folic acid deficiency', 'vitamin B9', 'vitamin B9 deficiency', and keywords relating to 'guideline, CPG'.

7.1.2 Inclusion criteria and study selection

The selection of CPGs was conducted by a single reviewer and checked by a second reviewer. Disagreements were settled through discussion. Swiss or international CPGs providing recommendation(s) on the appropriateness of folate testing, or on how management should be guided by test results, were eligible for inclusion. Only guidelines published in English, French, German or Italian were eligible for inclusion. Only CPGs that met all the following criteria, adapted from Graham (2011), were included:

1) contains the word 'guideline' or 'recommendation' in its title or introduction, or contains recommendations on folate testing
2) developed by at least 2 authors
3) developed based on a systematic search of the literature.[64]

Guidelines based only on expert opinion were excluded. Only guidelines published after 1 January 2017 were included because guidelines are outdated 5 years after publication.[64 65]

7.2 Results

In total, 5 guidelines met the inclusion criteria (***Table 11***).[66-70] No Swiss-specific guidelines on folate testing were identified. The included guidelines were published in the UK (k = 2),[68 70] North America (k = 2)[66 67] and Australia (k = 1).[69]

There were no general CPGs on folate testing; rather, the identified guidelines focused on the use of folate testing in 2 specific populations: patients undergoing bariatric surgery[66 67 69] and patients with suspected neurological deficiency as indicated by progressively worsening gait ataxia.[68] One guideline published by the Royal College of Pathologists (UK) specifically recommended against repeat folate testing of patients with a folate deficiency.[70] Importantly, although these guidelines were drafted based on a systematic review combined with a clinical review, many of the recommendations are either based on low quality evidence, or the evidence underpinning the recommendation was not reported. It is also important to note that only one guideline reported a threshold for identifying a clinically deficient patient, and none of the guidelines recommended a clear timing or frequency of testing and/or follow-up testing for folate deficiency.

It is worth noting that several other guidelines were identified in the searches, which did not meet the inclusion criteria (e.g. published prior to 1 January 2017 or based solely on expert opinion) but offer useful insights into the area. Most notably, the haematology guideline for the diagnosis and treatment of cobalamin and folate disorders,[23] which was excluded based on publication date, reiterates the fundamental issue with drafting guidelines in this area:

> *"These guidelines aim to provide an evidence-based approach to the diagnosis and management of cobalamin and folate disorders. However, such evidence, particularly in the form of randomized controlled trials, is lacking. As a result, these guidelines provide a pragmatic approach to the testing and treatment of cobalamin and folate disorders, with recommendations based, as far as possible, on the GRADE system….In the majority of situations, the recommendations inevitably rely more on clinical judgement and consensus than objective laboratory data."*[23]

Table 11 Clinical practice guidelines on serum folate testing

Author; year; country	Recommendation for folate testing	Strength of evidence	Diagnostic threshold
Queensland Health, 2021[69] Australia	•In overweight and obese pregnant women undergoing bariatric surgery, serum folate testing is recommended at preconception, first trimester, 2nd and 3rd trimester, and lactation (3 monthly) •Antenatal care for women with a raised BMI: consider screening for folate deficiency.	NR NR	NR
AACE/TOS/ASMBS/OMA/ASA, 2019[66 67] United States	All patients before and after any bariatric procedure; particular attention should be given to female patients of childbearing age. Patients who become pregnant following bariatric procedure; recommended testing every trimester.	NR Grade D (i.e. expert opinion)	RBC folate <305nmol/L (<135 ng/ml) [71]
O'Kane 2020 [67] Canada	In patients planning to undergo bariatric surgery: ***Preop recommendation*** •Haematinics: check full blood count including haemoglobin, ferritin, folate and vitamin B12 levels ***Postop recommendations*** •Check serum folate levels at regular intervals post-surgery •Consider the following frequency of monitoring of serum folate levels: 3, 6 and 12 months in the first year and at least annually thereafter so changes in status may be detected ***In cases of deficiency*** •Check and treat for vitamin B12 deficiency before initiating folic acid treatment to avoid precipitation of subacute combined degeneration of the spinal cord •Treat folic acid deficiency using NICE CKS: anaemia—B12 and folate deficiency. Folic acid 5 mg orally daily for a minimum of 4 months is recommended and further investigations if there is suspicion of malabsorption ***Pregnancy*** •Pregnant women, following bariatric surgery, should undergo nutritional screening during each trimester. This should include ferritin, folate, vitamin B12, calcium, vitamin D, vitamin A	 Grade B EL 2 (1+ to 4)†* Grade B EL 2 (1+ to 2−)†* GPP (i.e. expert opinion) Grade D EL 4† (i.e. expert opinion) Grade D EL 4† (i.e. expert opinion) Grade D EL 4 (i.e. expert opinion)	NR
NICE, 2019[68] United Kingdom	Adults with gradually progressive unsteady gait (gait ataxia)	NR	NR
Royal College of Pathologists, 2021[70] United Kingdom	'Repeat measurement of vitamin B12 and folate is unnecessary in patients with vitamin B12 and folate deficiency'	Level of evidence – D (i.e. case reports, case series of expert opinion)	NR

Abbreviations
AACE = American Association of Clinical Endocrinologists; **ASA** = American Society of Anaesthesiologists; **ASMBS** = American Society for Metabolic & Bariatric Surgery; **BMI** = body mass index; **CKS** = clinical knowledge summary; **EL** = evidence level; GPP = good practice point (recommended best practice based on the clinical experience of the guideline development group; **NICE** = National Institute for Care and Effectiveness; **NR** = not reported; **OMA** = Obesity Medicine Association; **RBC** = red blood cell; **TOS** = The Obesity Society
Note

†Grade B recommendation = A body of evidence including studies rates as 2++, directly applicable to the target population and demonstration overall consistency of results; or extrapolated evidence from studies rated as 1++ or 1+' Grade D recommendation = Extrapolated evidence from studies rates as 2++ evidence level 3 or 4; or extrapolated evidence from studies rated as 2+; Evidence level 2 (1+ to 4) = well conducted meta-analyses, systematic reviews or RCTs with low risk of bias to expert opinion; Evidence level 2 (1+ to 2-) = well conducted meta-analyses, systematic reviews or RCTs with low risk of bias to case-control or cohort studies with a low risk of confounding or bias and a moderate probability that the relationship is not casual; Evidence level 4 = expert opinion.
*The evidence rating for this recommendation is strongly questioned as supporting evidence is not clearly reported and the level of evidence supporting the specific recommendation for folate testing is unclear.

8 Budget impact analysis

Summary statement budget impact assessment

The clinical evaluation found insufficient evidence relating to health outcomes to undertake cost-effectiveness or cost - utility analyses comparing supplementation based on folate testing to supplementation without testing. A budget impact assessment was undertaken to estimate folate testing and treatment payer costs in Switzerland over the next 5 years. The costs of serum and blood cell folate tests increased from CHF 7.9 million in 2016 to CHF 11.7 million in 2020 and the value of folic acid treatment has increased from CHF 6.9 million in 2017 to CHF 7.7 million in 2021. Growth rates of testing and treatment volumes over the last 5 years were used to project costs for 2022–2026. Based on these assumptions, it is forecast that the combined cost of folate testing and treatment will increase from CHF 22.2 million in 2022 to CHF 30.8 million in 2026.

8.1 Methods

Value for money analysis of folate testing and treatment was captured in the ***HTA protocol*** by 2 key questions. The first, is whether supplementation based on folate testing had superior cost-effectiveness or cost-utility compared to supplementation without testing. It was noted that outcomes would be evaluated by considering existing published literature where available, and a *de novo* economic evaluation would be conducted if direct test-to-health-outcomes evidence existed. The clinical evaluation found insufficient evidence relating to health outcomes to undertake these types of economic evaluations.

The second key question involved estimation of the annual budgetary impact of folate testing and supplementation. It was decided that this analysis would be conducted regardless of whether a *de novo* economic evaluation was undertaken. The following sections present the budget impact of folate testing and treatment in Switzerland over a projected 5-year period. The current budget impact is initially outlined, followed by a projection from 2022–2026. No evidence or applicable clinical practice guidelines (see ***Section 7***) were found that could be used to inform different budget impact scenarios, such as limiting coverage to specific indications.

Literature searches of clinical practice guidelines on serum folate testing (see ***Section 7***) identified guidance associated with target populations, such as overweight and obese pregnant women prior to bariatric surgery[66 67 69] and adults with gradually progressive unsteady gait.[68] Guidance also indicated

that repeat measurement of vitamin B12 and folate is unnecessary in patients with vitamin B12 and folate deficiency.[70] It is difficult to use guidance associated with these target populations as part of scenarios for restricting folate testing and supplementation. They are mostly guidelines for managing risk factors associated with deficiency, rather than folate testing guidelines.

8.2 Results: Current budget impact

There are currently no limitations on mandatory insurance coverage of folate tests.[1] Serum and blood cell folate tests are reimbursed through mandatory health insurance in Switzerland. A total of 738,164 folate serum tests were ordered in 2020 and 96,445 blood cell tests in the same year. The combined total of tests was 834,609 in 2020, which is a substantial increase (47%) in the total number of tests since 568,416 in 2016 (See ***Table 12***). Most tests are billed by laboratories, which accounted for 86% of claims over this period.

There is limited information about the proportion of folate tests undertaken for different indications across Switzerland. Clinical feedback during the assessment suggested that testing related to endocrinology, cardiovascular, haematology, oncology, nutrition, transplantation, and internal medicine accounted for large proportions of folate testing in hospitals, and pathological risk factors (e.g., malignancy, haemolytic anaemia, coeliac disease, short bowel syndrome, inflammatory bowel syndrome etc.), alcoholism, and bariatric surgery patients in general practice. Given data limitations - along with the large numbers of groups that are tested; wide spread nature of indications and referrers – no quantification of test use by patient group has been calculated.

Table 12 Numbers of folate tests in Switzerland, 2016–2020

Year	2016	2017	2018	2019	2020
Folat, Serum (317.1329.00)					
Doctors	3,071	4,422	4,544	3,456	2,025
Hospitals	64,623	79,790	85,833	97,617	89,439
Laboratories	442,882	551,322	624,020	666,461	646,649
Other	12	13	20	35	51
Subtotal	**510,589**	**635,547**	**714,417**	**767,569**	**738,164**
Folat, Blood Cell (317.1330.00)					
Doctors	510	528	391	399	303
Hospitals	14,121	17,339	19,987	21,102	19,066
Laboratories	43,196	57,537	67,916	77,383	77,072
Other	0	1	2	2	4
Subtotal	**57,827**	**75,405**	**88,296**	**98,886**	**96,445**
Combined (Serum and Blood Cell tests)					
Doctors	3,581	4,950	4,935	3,855	2,328
Hospitals	78,744	97,129	105,820	118,719	108,505
Laboratories	486,078	608,859	691,936	743,844	723,721
Other	12	14	22	37	55
Total	**568,416**	**710,952**	**802,713**	**866,455**	**834,609**

The unit costs for the serum and RBC tests[1] are taken from 'Analysenliste' positions in 1 April 2021.[1] These costs are combined with volumes in ***Table 12*** to generate the costs in ***Figure 3***. It is evident that serum ('Blut') costs increased from CHF 6.7 million in 2016 to CHF 9.6 million in 2020. Blood cell folate test costs ('Erythrozyten') increased from CHF 1.2 million to CHF 2.0 million over the same period. The total cost for both types of tests amounted to CHF 11.7 million in 2020.

[1] Serum test (1329.00, 'Folat, Blut', cost of 13.1 tax points) and Blood cell (1330.00, 'Folat, Erythrozyten' cost of 21.0 tax points).

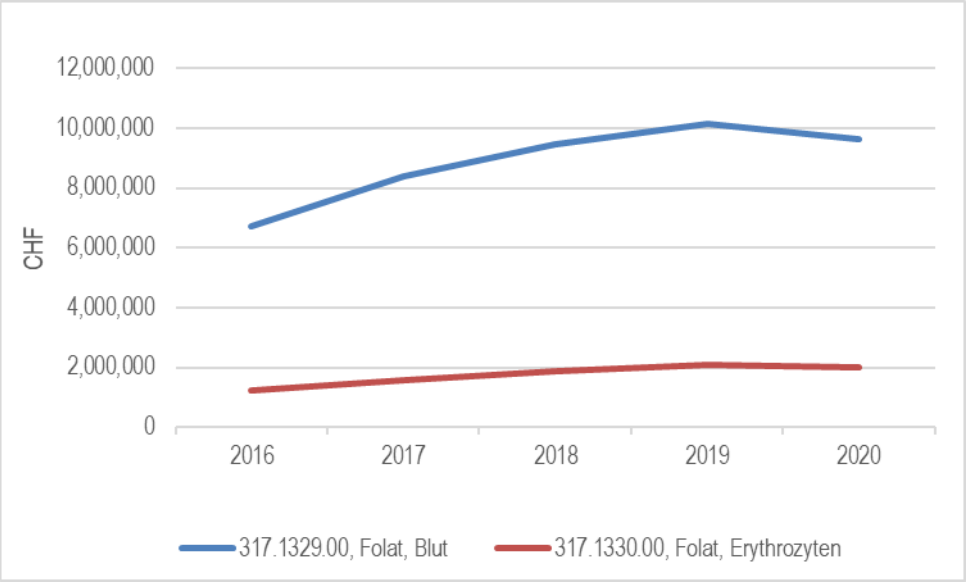

Figure 3 Costs of folate testing in Switzerland, 2016–2020

Source: SASIS AG[2]

Folic acid treatment and associated costs are presented using COGE© data in ***Table 13***. The 3 leading folic acid treatment products by volume were *Acidum folicum Streuli, Andreafol* and *Gyno Tardyferon*. Together they accounted for 85% of folic acid treatments across the 2017–2021 period. The leading product, *Acidum folicum Streuli,* accounted for nearly half (48%) of all folic acid treatments. The value of folic acid treatment has increased from CHF 6.9 million in 2017 to CHF 7.7 million in 2021.

Table 13 Folic acid treatment and costs in Switzerland, 2017–2021

Number of packs	2017	2018	2019	2020	2021
Dialvit	2,534	2,113	5,730	2,911	2,534
Gyno Tardyferon	83,989	76,091	73,368	75,805	54,837
Maltofer FOL	25,330	23,030	21,837	22,101	26,288
Duofer Fol	9,524	9,293	10,143	11,373	14,664
Andreafol	65,314	62,954	64,839	69,342	74,713
Fertifol	5,496	4,774	4,591	4,708	4,538
Acidum folicum Streuli	179,402	178,770	186,120	196,738	221,158
Drossafol	12,333	10,401	10,280	10,178	11,272
Metofol	963	1,096	1,290	1,437	1,631
Cernevit	1,669	1,937	2,673	8,224	3,642
Total	**386,554**	**370,459**	**380,873**	**402,817**	**415,277**
CHF					
Dialvit	74,765	71,568	75,998	78,539	80,348
Gyno Tardyferon	1,697,919	1,619,791	1,567,947	1,601,659	1,130,365
Maltofer FOL	667,960	625,042	595,119	604,442	728,932
Duofer Fol	273,583	271,480	293,718	335,140	422,716
Andreafol	1,549,725	1,542,423	1,604,175	1,720,163	1,871,455
Fertifol	134,718	115,252	83,579	83,066	79,852
Acidum folicum Streuli	2,295,332	2,442,337	2,566,520	2,748,705	3,017,419
Drossafol	131,382	141,095	144,378	144,830	159,971
Metofol	11,980	13,804	16,510	18,303	21,419
Cernevit	96,721	126,735	161,585	156,851	178,830
Total (CHF)	**6,934,084**	**6,969,527**	**7,109,528**	**7,491,699**	**7,691,306**

Abbreviations
CHF = Swiss franc.
Source: Tarifpool: © SASIS AG – Datenaufbereitung: © COGE

8.2.1 Assumptions in the budgetary impact analysis

Folate testing and treatment has increased in Switzerland over the last 5 years. Future projections outlined in ***Table 14*** include the growth rates observed over the last 5 years. In the case of folate testing, serum tests are assumed to increase by 10% per year; blood cell folate tests by 14% per year. Based on these assumptions, the number of serum tests increases from 893,179 in 2022 to 1,307,703 in 2026. Blood cell tests increase to 211,694 by 2022. Estimates for 2022–2026 are presented in ***Table 14***. The overall projected costs of testing increase from CHF 14.2 million in 2022 to CHF 21.5 million in 2026.

Acidum folicum Streuli and *Andreafol* folic acid treatments are estimated to increase by 8% and 2% per year, respectively, based on reported growth over the last 5 years. *Gyno Tardyferon* treatments are assumed to decrease by 8% per year based on the numbers over the last 5 years. Other treatments are assumed to grow by 3% per year, based on 5 years of data. It is estimated there will be 525,887 folic acid treatments in 2026 based on these assumptions. The projected payer cost of folic acid treatment

for Switzerland increases from CHF 7.9 million in 2022 to CHF 9.3 million in 2026. The combined cost of testing and treatment will increase from CHF 22.2 million in 2022 to CHF 30.8 million in 2026.

Table 14 Swiss folate testing and treatment projections, 2022–2026

Year		2022	2023	2024	2025	2026	Total
Numbers of tests							
Folat, Blut	No.	893,179	982,497	1,080,746	1,188,821	1,307,703	5,452,945
Folat, Erythrozyten	No.	125,340	142,888	162,892	185,697	211,694	828,510
Costs of tests							
Folat, Blut	CHF	11,657,850	12,823,635	14,105,999	15,516,599	17,068,259	71,172,342
Folat, Erythrozyten	CHF	2,631,307	2,999,690	3,419,647	3,898,397	4,444,173	17,393,214
Subtotal test costs	**CHF**	**14,289,157**	**15,823,325**	**17,525,646**	**19,414,996**	**21,512,432**	**88,565,556**
Folic acid treatments							
Gyno Tardyferon	No.	50,397	46,317	42,567	39,121	35,953	214,355
Andreafol	No.	76,452	78,232	80,053	81,917	83,824	400,477
Acidum folicum Streuli	No.	239,770	259,949	281,826	305,543	331,257	1,418,345
Other	No.	66,506	68,501	70,556	72,673	74,853	353,091
Total	**No.**	**433,126**	**452,999**	**475,002**	**499,254**	**525,887**	**2,386,268**
Costs of folic acid treatment							
Gyno Tardyferon	CHF	1,038,846	954,736	877,437	806,396	741,107	4,418,522
Andreafol	CHF	1,915,023	1,959,606	2,005,227	2,051,910	2,099,679	10,031,445
Acidum folicum Streuli	CHF	3,271,356	3,546,665	3,845,143	4,168,739	4,519,569	19,351,473
Other	CHF	1,722,230	1,773,897	1,827,113	1,881,927	1,938,385	9,143,551
Subtotal folic acid treatment costs	**CHF**	**7,947,455**	**8,234,904**	**8,554,920**	**8,908,972**	**9,298,740**	**42,944,991**
Total costs	**CHF**	**22,236,613**	**24,058,230**	**26,080,566**	**28,323,968**	**30,811,172**	**131,510,547**

Abbreviations
CHF = Swiss franc.

9 Ethical, legal, social and organisational (ELSO) issues

Summary statement ethical, legal, social and organisational issues

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A total of 8 studies were identified that addressed various ethical, social and organisational aspects of folate testing. No studies reporting legal issues were identified. Most studies identified addressed organisational issues relating to methods of test restriction; however, none discussed the implications of reducing test availability, including how folate deficiency would be diagnosed by physicians in the absence of a folate test. Ethical issues identified included potential adverse events from taking folic acid supplements if not actually folate deficient, which could occur with a false positive test or an incorrect diagnosis by a clinician in the absence of a test. Studies on social issues were in relation to physicians' opinions and concerns regarding an initiative to reduce folate test orders, which were mostly supportive, and citizens' opinions on test disinvestment.

9.1 Literature search strategy

9.1.1 Databases and other sources

A literature search for studies reporting ethical, legal, social and organisational issues associated with folate testing was conducted in PubMed and Embase (OVID). In addition, studies included in the clinical section of this report were pearled for citations relating to these auxiliary domains. Grey literature searches were also conducted on specialty and HTA websites (***Table 26*** and ***Table 27***, ***Appendix A***) using combinations of the keywords 'folate', 'folic acid', 'folacin', 'folate deficiency', 'folic acid deficiency', 'vitamin B9', 'vitamin B9 deficiency' and 'test*'.

9.1.2 Inclusion and exclusion criteria

Table 8 (***Section 4.5***) describes the populations, interventions and comparators that were applied.

9.2 Data extraction, analysis and synthesis

9.2.1 Study selection

The targeted search for studies reporting on ethical, legal, social and organisational issues associated with folate testing was conducted by a single reviewer. A PRISMA chart was not provided owing to the use of systematic and non-systematic searches.

9.2.2 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. Data of interest included:

- study information: country, year, study design, research question, inclusion/exclusion criteria
- demographic information: number of participants, age, sex, comorbidities, indication for testing
- outcomes of interest: results relating to ethical, legal, social or organisational issues
- any noteworthy features, limitations or differences in the study.

9.2.3 Data synthesis

The results of the included studies were described narratively, per 'ELSO' domain.

9.3 Results

A total of 8 studies, published between 2015 and 2021, were identified for inclusion.[31 55 72-77] Of these, 4 were from Canada,[31 73-75] plus one each from Australia,[76] Saudi Arabia,[72] Turkey[77] and China.[55]

9.3.1 Ethical issues

Ethical concerns identified relate to the potential adverse events that can occur with folic acid supplementation. This is relevant in situations where a patient is given a supplement based on an 'incorrect' folate test diagnosis (i.e. false positive), and also if the test were disinvested and patients are incorrectly given a supplement based on a clinician's suspicion of folate deficiency when not deficient. An umbrella review that included meta-analyses of RCTs on folic acid supplementation reported that folic acid supplements are generally considered to be safe, but are associated with an increased risk of colorectal adenoma in the general population and prostate cancer in people with pre-existing diseases (***Table 15***).[55] The authors hypothesised that for the association between folate and increased risk of colorectal adenoma the most likely explanation is that undiscovered early precursor lesions might have existed in the mucosa of these patients and folate could have accelerated the proliferation and growth of the paraneoplastic lesions.[55] The review also found an association between periconceptional pregnancy supplementation and child asthma and wheezing and early pregnancy folic acid supplementation and child wheezing. However, it should be noted that these associations were from observational study data, and the likelihood of patients being given supplements unnecessarily is unclear owing to the limited diagnostic accuracy of folate testing.

Table 15 Characteristics of included study for ethical issues

Author, year; country	Population; sample size	Design; follow-up; setting; mandatory fortification	Aim of study	Results
Bo 2020[55]; China	Studies published from inception to 20 May 2018 which examined the association of folate with any health outcome; k = 108.	Umbrella review of meta-analyses; NR; NR; NR.	To evaluate the quantity, validity and credibility of evidence regarding associations between folate and multiple health outcomes.	108 articles reporting 133 meta-analyses of observational studies and 154 meta-analyses of RCTs were included in this umbrella review. Benefits of folic acid: reduced all-cause mortality and chronic disease rate (including birth/pregnancy outcomes, cardiovascular disease, neurological conditions). Adverse effects of folic acid: increased risk of prostate cancer, colorectal adenomatous lesions, child asthma and/or wheezing.

Abbreviations
NR = not reported; **RCTs** = randomised controlled trials.

9.3.2 Legal issues

No studies were identified that addressed legal issues for folate testing.

9.3.3 Social issues

Two studies reporting social issues were identified; one each reporting physician and citizen opinions on disinvestment of folate testing, respectively (***Table 17***).

Physician views on disinvestment from folate testing

One study, by Ismail (2019), described an initiative to reduce the number of folate tests ordered across 2 centres in Canada (described below in organisational issues) and provided some detail on the opinion of physicians on test restriction.[31] The study audited folate tests over a 18-month period and identified 111 physicians who had ordered 5 or more tests during this time. These physicians were contacted via email and informed of the centres' plan to restrict testing by requiring biochemist approval of all future folate tests. They were also given the opportunity to provide feedback. Only 38 physicians responded, 34 of whom did not have an issue with test restriction and 4 who voiced concerns for particular patient groups, including those with coeliac disease, gastric bypass or severe anorexia, and immigrants from countries where folic acid fortification does not take place. It is difficult to make assumptions based on this limited information; however, most physicians responding to the survey felt the changes to folate test ordering were appropriate.[31]

Citizen views on disinvestment from folate testing

One Australian study reported citizens' perspectives on disinvestment from publicly funded pathology tests.[76] Disinvestment of vitamin B12/folate was used to shape the debate and the forum was informed by a systematic review of B12/folate pathology test effectiveness and expert testimony. The forum consisted of 10 participants; half were men and ages ranged from 20 to 66 years. The citizens identified 7 criteria they deemed as important to inform the prioritisation of potential test disinvestment. Their general opinions relating to these criteria are summarised below (***Table 16***). A comparison of the criteria devised by the citizen forum and those devised by an expert group was made. There was considerable coherence between the 2 groups. The major differences being that the citizens did not consider equity issues, whilst the expert group did not consider the cost of the social and emotional impact of disinvestment on users and society.[55]

Table 16 Citizens' perspectives on criteria deemed important to inform the prioritisation of potential test disinvestment

Citizen criteria	Opinions expressed
Cost of test	Participants agreed that the cost of the test was a central point to consider for potential disinvestment. Emphasis was placed on high cost per procedure with little emphasis on cost by volume. Overall cost was an impetus for action.
Potential benefit	High potential for benefit was considered as worthy of funding. Equal value was generally given for quality of life and extension of life. The possibility of test exclusion was expressed where the condition is related to lifestyle choices.
Costs to the community of not testing	Attention was given to the ongoing cost to the community of doing nothing (financial cost to the health system and emotional cost to individuals and family).
Public good	Public good was frequently prioritised over individual benefit in many of the arguments raised by participants.
Alternatives to testing	The participants agreed that having alternatives to testing was an important criterion. One member suggesting that pre-emptive treatment may be a solution.
Disease severity	The severity of the illness played a crucial role in participants' understanding of whether a test should be funded.
Accuracy	Accuracy was included as one of 4 primary criteria to consider for disinvestment decisions but was rarely considered in isolation from the other criteria.

In addition to developing a set of criteria relating to disinvestment of tests, the participants discussed the nature of a disinvestment process. The authors noted that some participants questioned whether policymakers had the authority to make these clinical decisions or whether the responsibility should lie with general practitioners, either supported by a set guideline or monitored through auditing. Suggestions regarding potential ways of disinvesting included tighter guidelines or descriptors for administering the test along with education and treatment strategies that would avoid the need to test.[76]

Table 17 Characteristics of included studies for social issues

Author, year; country	Population; sample size	Design; follow-up; setting; mandatory fortification	Aim of study	Results
Ismail 2019[31]; Canada	RBC folate requests (inpatients and outpatients) from 2 centres from Oct 2015–Apr 2018; n = 3,299	Pre-test post-test study (case series); 10 months; London Health Sciences Centre and St Joseph's Healthcare London; Yes	Review RBC folate tests ordered over a 18-month period (Oct 2015–Mar 2017) and implement a quality improvement initiative to reduce test numbers. The initiative included identifying physicians who had ordered ≥5 folate tests in the previous 18 months and providing them with education (over email) and informing them of the institution's intention to limit testing moving forward. Physicians were given the opportunity to provide their feedback on this.	111 physicians were identified as ordering ≥5 tests and were contacted. 38 responded (34 with no major concerns and 4 with concerns for patient groups such as those with coeliac disease, gastric bypass, severe anorexia, or immigrants from countries without folic acid fortification).
Street 2015[76]; Australia	Random sample of participants drawn from a state-wide survey by an independent recruitment company; n = 10	Case study; NA; academic institution; Yes	To describe the use of a deliberative forum to explore citizen's perspectives on disinvestment of pathology tests, using folate and B12 testing as an example.	Citizens identified 7 criteria of importance: cost of the test; potential impact on individual health/capacity to benefit, potential cost to society; public good; alternatives to testing; severity of the condition; accuracy of the test. (Their perspectives on these are described in more detail in ***Table 16***). Citizens also questioned the "authority" of policymakers to make disinvestment decisions.

Abbreviations
NA = not applicable; **RBC** = red blood cell.

9.3.4 Organisational issues

There were 6 studies identified that discussed organisational issues relating to methods to restrict or reduce folate testing.[31 72-75 77] The characteristics and main findings of these studies are reported in ***Table 18***. It should be noted that whilst the studies discussed how restrictions to folate testing were implemented or could be implemented, none of the studies reported how physicians would make a clinical diagnosis of folate deficiency if folate testing was not available. The studies that implemented restrictions noted cost savings to their institutions relating to folate testing; however, the cost implications of using other tests in lieu of folate testing are not known. As a result, the organisational implications for clinical laboratories that may have to perform an increased number of other tests is also unknown.

Appropriateness of testing

Three studies suggested patient groups in which folate testing could be limited to avoid unnecessary testing.[31 72-74] Alkhaldy (2020) defined the clinical criteria for testing as the presence of macrocytic anaemia, pancytopenia or bicytopenia. In their study, less than 50% of folate tests ordered met these clinical criteria.[72] Both Gudgeon (2015) and Ismail (2019) suggested specific circumstances for folate testing were suspected severe nutritional deficiency or malabsorption.[31 73] Whilst making recommendations regarding specific circumstances or criteria for folate testing, the studies did not discuss how these recommendations could be implemented or monitored within a healthcare system.

Bundling of tests

Alkhaldy (2020) noted that folate tests tended to be bundled with vitamin B12 and ferritin testing, resulting in both increased costs and time loss. The authors suggested that informing physicians of the low incidence of folate deficiency and changing laboratory processes, such as storage of samples, may reduce unnecessary folate tests.[72]

Interventions used to restrict folate testing

Four studies described interventions to restrict folate test ordering; all of which resulted in significant reductions in test numbers, and associated costs, over their study period.[31 74 75 77] These are described in detail in ***Table 18*** and are briefly summarised below:

- Providing physicians with education regarding the low prevalence of folate deficiency and of evidence-based medicine practices.[31 77]
- Adding a 'pop-up window' to the computerised ordering system, which states that folate tests should only be ordered in cases of suspected severe nutritional deficiency or malabsorption and that approval of the test by an on-call biochemist was required.[31]
- Limiting the ability to request folate tests via the computerised ordering system to clinicians in gastroenterology and haematology departments only (other departments could only order via written or telephone request to the laboratory).[74]
- Complete removal of serum and RBC folate tests from standard laboratory requisition requests so that requests could only be made in writing.[75]
- Providing monthly laboratory-use reports to departments (including information on test numbers and costs per patient).[77]
- Rearranging computerised test ordering systems, such that frequently ordered tests were on the second page.[77]
- Forbidding the use of test panels.[77]

Table 18 Characteristics of included studies for organisational issues

Author, year; country	Population; sample size	Design; follow-up; setting; mandatory fortification	Aim of study; Intervention	Results	Conclusions
Alkhaldy 2020[72]; Saudi Arabia	Serum folate requests at single centre from Jul 2018–Jun 2019; n = 614	Retrospective chart review; NR; Aseer Central Hospital; Yes (flour)	Analyse the clinical appropriateness of ordering serum folate tests; NA	Most common indication (where reported) for testing was anaemia (51%). Types of anaemia were microcytic (63.5%), normocytic (33%) and macrocytic (3.2%). Concurrent tests = serum B12 (88%) and serum ferritin (83%) Low serum folate was observed in 2.8% of cases – rate of low folate was NS between anaemic and non-anaemic patients.	Criteria for serum folate testing were not met in approximately 50% of the tests performed. Bundling of tests (presumably to save time to diagnosis and multiple blood extractions) occurred; however, it is inappropriate to order multiple tests without considering aetiology of anaemia. Multiple tests increase costs and time lost. Education and laboratory protocols (such as storage of samples) could reduce unnecessary folate test requests.

Author, year; country	Population; sample size	Design; follow-up; setting; mandatory fortification	Aim of study; Intervention	Results	Conclusions
Gudgeon 2015[73]; Canada	RBC folate requests at 3 centres from 1 Jan–31 Dec 2010; n = 2,563	Retrospective chart review; NR; 3 academic hospitals (Toronto General Hospital; Toronto Western Hospital; Princess Margaret Hospital); Yes	Review inpatients with folate deficiency to determine the indication for testing and aetiology of deficiency; NA	4/2,563 (0.16%) of tests were folate deficient (<254 nmol/L [<112 ng112ng/ml]) Of the 4 patients with folate deficiency, one test result was considered an error and the aetiology of the remaining 3 included alcohol abuse (n = 1), malabsorption syndrome (n = 1) and decreased oral intake due to schizophrenia (n = 1). All 3 patients with folate deficiency were anaemic but only 2/3 had elevated corpuscular volume. No blood films suggested megaloblastic anaemia. At a cost of $12.54 per test, $32,140 per year could be saved at this institution if folate testing was restricted.	Since the incidence of folate deficiency in inpatients is very low, significant cost savings could be achieved by restricting folate testing. Additional savings could be achieved if outpatient testing was also restricted. Specific circumstances for folate testing may include malabsorption and starvation. Folate deficiency as an aetiology of anaemia should be de-emphasised in Canadian medical training given its very low incidence (in countries where mandatory fortification occurs).

Author, year; country	Population; sample size	Design; follow-up; setting; mandatory fortification	Aim of study; Intervention	Results	Conclusions
Ismail 2019[31]; Canada	RBC folate requests (inpatients and outpatients) from 2 centres from Oct 2015–Apr 2018; n = 3,299	Pre-test post-test study (case series); 10 months; London Health Sciences Centre and St Joseph's Healthcare London; Yes	Review RBC folate tests ordered over an 18-month period (Oct 2015–Mar 2017) and implement a quality improvement initiative to reduce test numbers. The initiative included identifying physicians who had ordered ≥5 folate tests in the previous 18 months and providing them with education (over email). This included information on the low incidence of folate deficiency in the previous 18 months and the institution's intention to limit testing moving forward. Physicians were given the opportunity to provide feedback. Three months later, introduction of a pop-up within the computerised test ordering system stating biochemist approval was required for folate testing took place.	Initial retrospective chart review identified 0 cases of folate deficiency from the 3,019 tests ordered in the 18-month period. 111 physicians were identified as ordering ≥5 tests and were emailed education. 38 responded (34 with no major concerns and 4 with concerns for patient groups such as those with coeliac disease, gastric bypass, severe anorexia, or immigrants from countries without folic acid fortification). The number of tests post-education was 255 (none of which identified folate deficiency) and the number of tests post-pop-up was 25 (2 of which indicated folate deficiency according to the testing site's reference interval but not according to WHO's definition).	Overall, the mean number of tests per month pre-intervention was 168 (20 SD). This was significantly reduced to 128 (1 SD) after physician education ($p < 0.01$) and further reduced to 3 (2 SD) after introduction of the biochemist approval process ($p < 0.01$). Monthly costs of testing reduced by 95% following the implementation of the centres' intervention. Even taking into consideration the increased cost associated with moving testing off-site.

Author, year; country	Population; sample size	Design; follow-up; setting; mandatory fortification	Aim of study; Intervention	Results	Conclusions
MacMillan 2018[74]; Canada	RBC folate tests at a large academic healthcare network from Jan 2010–Dec 2016; n = 21,402 tests	Pre-test post-test study (case series); total reduction in test number was estimated for 43 months post-implementation of the intervention; large academic healthcare network in Toronto, Canda; Yes	The ability to request RBC testing was restricted in the computerised physician order entry part of an electronic health record to clinicians in gastroenterology and haematology and was removed from other physicians' computerised order entry screen in June 2013. Clinicians who were not specialist in haematology or gastroenterology could still request RBC folate via paper requisition or telephone call to the laboratory.	In the pre-intervention period 57/20,214 (0.28%) of RBC folate tests met the criteria for deficiency compared with 6/1,188 (0.51%) post-intervention. Assuming a stable rate of RBC folate deficiency over time this suggest that 1.25 cases per month of RBC deficiency were possibly missed due to the intervention, assuming the test is correct. RBC folate test numbers decreased by 94.4% from a mean of 493.0 (SD 48.0) tests per month before the intervention to 27.6 (SD 10.3) test per month after the intervention ($p < 0.001$).	Restricting RBC folate tests resulted in a large and sustained reduction in RBC folate testing with significant cost savings and without significant clinical impact on the diagnosis RBC deficiency.

Author, year; country	Population; sample size	Design; follow-up; setting; mandatory fortification	Aim of study; Intervention	Results	Conclusions
Mohammed-Ali 2021[75]; Canada	RBC and serum folate tests from 6 family medicine units from Sept 2018–Sept 2019; n = NR (study looked at multiple tests and the number of folate tests was not reported)	Pre-test post-test study (case series); 6 months post-implementation of the intervention; 6 family medicine units; Yes	To improve test utilisation by 6 family medicine units by revising standard laboratory requisition requests. Historical laboratory requisitions were reviewed and modified by a steering committee of family medicine physicians, laboratory medicine specialists and a quality improvement specialist. Decisions were made by consensus. Tests were removed from the requisition if there was evidence in the literature of overuse, if they were outdated or if there was consensus that they were infrequently needed in a family practice. Tests that were removed from the requisition remained orderable by longhand (both serum and RBC tests were removed). Outpatient monthly laboratory test volumes for 20 tests were extracted. The volume of tests pre- and 6 months post-implementation were compared.	A significant reduction in the volume of folate tests ordered was observed (serum folate, 87.2% reduction; $p < 0.001$; RBC, 76.8% reduction, $p < 0.001$). The monthly cost of serum folate testing dropped by 87.1% and the monthly cost of RBC testing dropped by 76.9%.	Revision of a family medicine laboratory requisition was an effective strategy to decrease unnecessary RBC testing, resulting in significant cost savings.

Yilmaz 2016[77]; Turkey	Folate (type NR) and other tests from a training and research hospital in Turkey n = NR	Pre-test post-test study (case series); one year follow-up; training and research hospital; Unknown	To define and process strategies to rationalise laboratory use in a training and research hospital and calculate the potential savings in costs. A review of how various laboratory tests were used and whether their use was appropriate according to the guidelines or literature. A systematic literature review was also performed to identify strategies used to improve laboratory efficiency. The barriers which could block the process during its implementation and possible solutions were discussed by a committee. Strategies implemented included: 1) Education of clinicians about evidence-based practice through hospital meetings. 2) A laboratory use report including test ordered per patient and costs was sent to each department monthly. 3) The computerised test ordering page was rearranged. Tests ordered more than expected or misused	In the 12 months following implementation of the strategies in March 2013, there was a 67.7% reduction in. the number of folate tests ordered	Hospital-based committees that include laboratory professionals and clinicians can lead to a standardised approach to test use that can help clinicians considerably reduce laboratory costs through appropriate use of laboratory tests.

Author, year; country	Population; sample size	Design; follow-up; setting; mandatory fortification	Aim of study; Intervention	Results	Conclusions
			were placed on the second page. 4) The use of test panels was forbidden. 5) Prompting notes were integrated into the computerised test ordering page.		

Abbreviations

CRP = C-reactive protein; **eGFR** = estimated glomerular filtration rate; **ESR** = erythrocyte sedimentation rate; **GPs** = general practitioners; **MCV** = mean corpuscular volume; **NA** = not applicable; **NR** = not reported; **RBC** = red blood cell; **SD** = standard deviation; **WHO** = World Health Organization.

10 Additional issues

10.1 Defining folate deficiency

A complicating factor regarding the diagnostic accuracy of folate testing, as noted in the guidelines by Devalia (2014),[23] is that there is no consensus on the cut-off to define deficiency (***Section 2***).[23] Specifically, Devalia (2014)[23] states:

> *'There is no clear consensus on the level of serum folate that indicates deficiency. Conventionally, clinicians have used serum folate lower than 7 nmol/l (3 µg/l) as a guideline because the risk of megaloblastic anaemia greatly increases below this level. However, there is a sizeable "indeterminate zone" (between approximately 7 and 10 nmol/l [3 and 4.5 µg/l]). Therefore, a low serum folate level should be taken as suggestive of deficiency rather than as a highly sensitive diagnostic test.'*[23]

10.2 Test variability

Adding to the uncertainty regarding the diagnostic accuracy of folate testing are several publications noting variability in the results obtained with different commercially available competitive protein binding assays used for folate testing. A recent comparison of 4 different immunoassays and a rapid isotope dilution liquid chromatography tandem mass spectrometry assay reported that the consistency of results among the evaluated assays for serum folate was worrisome. It was further stated that most method differences among the evaluated assays exceeded the acceptable quality specifications.[35] This issue has been highlighted in a recent review by Ferraro (2022)[28] who noted critical issues regarding marketed serum folate assays, specifically that the assessment of folate status changes depending on the assay used. They reported that folate thresholds as a measure of overt folate deficiency may vary by approximately 50% across different assays and/or by using the WHO recommended value as baseline.[28] While the type of folate assays used in Switzerland to determine serum or RBC folate are not reported on the FOPH List of Analyses, it is reported that these competitive protein binding assays are the type most commonly used. As summarised by Ferraro (2022),[28] inaccurate test results can result in higher healthcare costs and increased risks to patients by causing additional tests to be conducted or unnecessary supplementation. In situations where folate status is overestimated, the patient may not be prescribed folic acid supplementation when it is required. The authors suggest the adoption of method-dependent thresholds is crucial to personalise folic acid supplementation and improve the risk-benefit ratio.[28]

10.3 Appropriateness of testing

Owing to concerns regarding the appropriateness of folate testing, several recent studies have looked at either the appropriateness of folate test requests and/or the implementation of different strategies to ensure appropriate test requests.[31 72 74] These studies have been summarised in ***Table 18*** (***Section 9***). It should be noted that these studies were from countries that have mandatory folic acid fortification.

In addition, a recent review on folate testing in European countries by Ferraro (2017)[8] note that folate tests are inappropriate when there is no specific risk condition/suspicion for folate deficiency/inadequate status, the subject is undergoing or has recently undergone folic acid supplementation, and for retesting at 3–6 months to evaluate the actual correction of the deficit after intake discontinuation.[8]

Limiting folate testing based on specific criteria might be viewed as a method for restricting unnecessary folate testing; however, basing testing on clinical indications (e.g. haematocrit, mean corpuscular volume, haemoglobin, transferrin) does not appear to be useful. Latif (2004) (***Section 6.6***) reported that clinical indications are not helpful in determining folate-deficient patients. In their chart review comparing indications for testing among individuals with low serum folate levels values with those with normal levels, no differences in the indications for testing were found between the 2 groups.[50] Similarly, Vinker (2013) (***Section 6.6***) concluded that neither laboratory findings nor clinical findings were capable of distinguishing folate-deficient patients from a separately matched control group of patients.[54]

If folate testing is limited or withdrawn, clinicians might choose to order the homocysteine test, which is on the Analysenliste, in order to help determine if a patient is folate deficient. It should be noted this test is more expensive than serum and RBC folate tests.

10.4 Patient outcomes potentially associated with folate testing

Due to the paucity of data on the diagnostic accuracy of folate testing and a reported failure of test results to impact management decisions, a formal assessment of patient outcomes resulting from a change in management due to folate testing could not be undertaken. However, the implications of the intervention and comparator on patient outcomes has been considered and are discussed here. It is important to note that while the broad implications of each outcome have been discussed, it is not possible to quantify the relative ratio of patients who would fall into each group; therefore, the relative safety and effectiveness of the intervention and comparator cannot be assessed. The differences in approach between the intervention and comparator are summarised in ***Table 19***.

Table 19 Description of treatments approaches for intervention and comparator strategies for patients indicated for a folate test

Treatment	Intervention – folate testing and management dependent on results	Folic acid supplementation without prior testing
Folic acid supplement	Patients with a true positive or false positive test result [a]	All patients[b]
No folic acid supplement	Patients with a true negative or false negative test result	No patients[b]

Notes

a = change in management data indicate not all patients with a positive result will receive a supplement; **b** = actual rates of supplementation in this group are not known.

10.4.1 Intervention: Folate testing

True positives

Under the intervention strategy, patients identified as having a folate deficiency would be provided with a folic acid supplement and would therefore receive the benefits of folic acid supplementation and be exposed to the associated risks. However, we note that the change in management suggests that, even with a clinical suspicion of folate deficiency to warrant a test and a subsequent result indicating deficiency, only ~50% of patients receive a supplement.[47-54]

Effectiveness of folic acid supplementation

In their umbrella review, Bo (2020) found high- or moderate-quality (RCT) evidence that folic acid supplementation was associated with a number of improved health outcomes, including a reduction in:

- terminations due to foetal abnormalities (RR 0.29, 95% CI 0.15, 0.57)
- megaloblastic anaemia (RR 0.26, 95% CI 0.14, 0.46)
- neural tube defects (RR 0.33, 95% CI 0.18, 0.62)
- cardiovascular disease (among patients with pre-existing disease) (RR 0.94, 95% CI 0.90, 0.99)
- liver toxicity (patients receiving methotrexate) (RR 0.20, 95% CI 0.11, 0.36)
- gestational hypertension/preeclampsia (RR 0.62, 95% CI 0.44, 0.89)
- Hamilton Depression Rating Scale score (RR 0.01, 95% CI 0.00, 0.51)

Risks of folic acid supplementation

As described under safety of folic acid supplementation (***Section 6.7***).

False positives

Patients incorrectly diagnosed as being folate deficient would be exposed to the risks of folic acid supplementation described above.

True negatives

Patients correctly identified as not having folate deficiency would avoid unnecessary supplementation.

False negatives

Patients incorrectly identified as having normal folate levels may experience a delay to diagnosis, or may not be diagnosed, and are subject to the associated risks of continued folate deficiency.

Natural history of folate deficiency

No studies investigating the impact of a delayed diagnosis or natural history of folate deficiency were identified.

However, it is established that folate deficiency is associated with serious health conditions:

- Folate deficiency leads to an increase in the risk of neural tube defect and the use of pre-conception supplements can reduce this risk by 40–80%.[78]
- Untreated folate deficiency leads to megaloblastic anaemia and pancytopenia.[79 80]
- Folate is known to lower plasma homocysteine, which is associated with cardiovascular disease and stroke.[10 78]

10.4.2 Comparator: Supplementation without prior folate testing

Under the comparator as defined for this HTA, it is assumed all patients with a suspicion of folate deficiency would be recommended to take a supplement; however, no evidence was identified that explored the likelihood of this occurring in clinical practice. Management data suggests most patients referred for a folate test will have normal folate levels. Therefore, these patients may be prescribed folic acid they don't require, and will be exposed to the potential harms of folic acid supplementation.

11 Discussion

The main aim of this HTA report was to determine the benefits and harms of folate testing prior to folic acid supplementation in an asymptomatic general population and in patients with suspected folate deficiency, compared to supplementation without prior folate testing. In addition, the ethical legal and social issues associated with folate testing and the financial implications of removing folate testing were examined. The 2 types of folate tests evaluated were serum and RBC folate, as these are currently reimbursed by mandatory health insurance in Switzerland.

No direct evidence on folate testing was identified, necessitating a linked evidence approach. Overall, there is a lack of evidence on folate testing to inform policy decision-making, with one outdated study on the diagnostic accuracy and no studies on change in management. Adding to the uncertainty regarding folate test accuracy is the lack of consensus regarding a cut-off value to define folate deficiency, the fact that the assessment of folate status seems to change in relation to the analytical platform used, and an absence of CPGs informing the recommended use of folate testing in the studied populations.

11.1 Comparison to prior HTA reports on folate testing

Four HTA reports were identified by grey literature searches and pearling, 3 from Canada and one from Australia. The most recent of these, published in 2022 by the Canadian Agency for Drugs and Technologies in Health (CADTH)[81], is an update of their 2015 report[82] on folate testing. Another recent Canadian HTA, published in 2020, is by the National Institute of Excellence in Health and Social Science (INNESS)[83] on erythrocyte folate assay. The Australian Medical Services Advisory Committee (MSAC) published their Medical Benefits Schedule (MBS) review of folate testing in 2014.[84] A report by the Ontario Health Technology Advisory Committee (OHTAC, Canada), published in 2013, was described in the above HTAs but could not be accessed; it is also not clear if this is an HTA. The 3 HTAs which could be retrieved are summarised below:

- **CADTH (2022; 2015):** The 2015 Rapid Response Report reviewed both database and grey literature evidence from January 2010 to June 2015. The 2022 HTA search dates were January 2015 to February 2022 (with studies identified in the 2015 report excluded). The 2022 HTA was unable to identify any relevant literature regarding the diagnostic accuracy, clinical utility or cost-effectiveness of serum folate testing in people suspected of folate deficiency. The review also failed to identify any evidence-based CPGs on the use of serum folate testing. CADTH concluded that in the absence of evidence, conclusions could not be drawn, and the creation of guidelines was precluded.[81 82]

- **INNESS (2020):** A 2020 report by INNESS assessed the clinical utility of RBC folate testing. Databases were searched from January 2015 to January 2019 for relevant studies, whilst searches for CPGs were conducted from 2010 to July 2019. A decision was made by clinical experts, taking into consideration the scientific evidence, that some clinical situations require measurement of RBC folate (i.e. when serum test results are ambiguous or when it is suspected that recent nutritional intake may be masking deficiency when serum testing alone is used). As such, it was recommended that the ability to order this analysis by means of a special prescription or shipment outside Quebec should be kept. The report did not discuss the diagnostic accuracy of the test.[83]
- **MSAC (2014):** This review searched both database and grey literature from January 2002 to June 2013 to determine the appropriate indications for folate testing, if folate testing improves health outcomes, the risks/harms of folate testing and if test quality varied between testing platforms. MSAC concluded that few guidelines recommending folate testing were identified. Of the 2 that were, one recommended folate testing as part of a basic dementia screening process and the other did not recommend folate testing in chronic fatigue/myalgic encephalomyelitis patients unless a full blood count and MCV showed macrocytosis. The review concluded there was no prospective evidence regarding the clinical utility of folate testing in any patient population or evidence regarding the risks/harms of folate testing. Based on poor evidence, the authors also concluded that serum folate appears the best test option with regards to cost and clinical information. Additionally, given the exceptionally low utility of serum folate testing, the costs associated with them are excessive.[84]

Overall, the findings of these existing HTAs are consistent with those of the current report. There is a lack of evidence available to determine the effectiveness and safety of folate testing.

11.2 Limitations

Limitations of the diagnostic accuracy evidence

The main limitation to the evidence is that it is based on one study that used a radioassay to determine RBC folate, which is reportedly no longer used by clinical laboratories today (personal comment, haematologist). Thus, the results from this study are unlikely to be representative of the accuracy of folate testing occurring in Switzerland today. Further, it had a mostly unclear-to-high risk of bias due to the paucity of reporting within the study.

Limitations of the change in management evidence

There is uncertainty regarding the applicability to the Swiss population of the studies reporting on management decisions following folate testing, particularly with respect to the percentage of patients

that are diagnosed as folate deficient, as 7 of the 8 studies were from countries with mandatory folic acid fortification. Furthermore, the serum folate thresholds used in the studies to mark folate deficiency varied from <4.8 nmol/L (<2.1 ng/ml) to <10.8 nmol/L (<4.8 ng/ml), whilst the RBC thresholds were 175 ng/ml (397 nmol/L) in 2 studies and 155 ng/ml (351 nmol/L) in the other. Another uncertainty of the study applicability relates to the setting of the studies. Six of the 7 studies were conducted in a hospital setting, one was based in primary care clinics, and the remaining study was a mixture of hospital and general practitioner request data. SASIS data shows that most folate tests ordered in Switzerland are coded as being claimed by laboratories. It is unclear from where most of these have originated (e.g. originally ordered by general practitioner or by hospitals). The management of patients treated by specialists in a hospital setting may be different to what would occur in a general practice setting. In addition, whether patients would be managed differently by Swiss hospitals and general practitioners (i.e. more or all patients diagnosed as deficient given supplementation) is not known. All the studies were retrospective case series and thus at high risk of bias based on their study design; however, all were consistent in their findings of low rates of folate deficiency being detected and low rates of supplementation even when deficiency was identified.

Limitations of the safety evidence

There is some uncertainty regarding the significant finding for prostate cancer as it was driven by results from a single study which might indicate heterogeneity in the included populations. The colorectal adenomatous lesion finding was in patients who had been on folate supplementation for over three years and the meta-analyses was deemed to be of very low quality as assessed by GRADE. The association of folic acid supplementation prior to and during pregnancy with child wheezing and asthma was based on observational studies and thus should be interpreted with caution.

Limitations of the budget impact analysis

A budget impact analysis has been undertaken to estimate folate testing and treatment payer costs in Switzerland over the next 5 years. Projected growth rates in the volumes of testing and treatment were based on historical annual volume growth. A more accurate calculation would be based on epidemiological data outlining the size of subpopulations of Swiss patients who could avail testing and folic acid treatment, along with the potential for future uptake being calculated. The clinical evaluation has found insufficient evidence relating to health outcomes to undertake economic analyses that compare supplementation based on folate testing to supplementation without testing. This lack of evidence, along with an absence of applicable clinical practice guidance, has limited the identification of appropriate subpopulations that could be used in budget impact delisting scenarios.

12 Conclusions

Due to a lack of evidence, this report is limited in its ability to make conclusions regarding the benefits and harms of folate testing prior to folic acid supplementation in an asymptomatic general population, and in patients with suspected folate deficiency due to the presence of symptoms, underlying medical disorders, or external factors, compared to supplementation without prior testing. This finding is consistent with previous HTAs. No ongoing clinical trials were identified that are likely to fill this evidence gap in the near future.

Irrespective of the lack of diagnostic accuracy data regarding folate testing, and the uncertainty raised in the literature about folate testing, it appears that test results have limited influence on patient management. However, it should be noted that this conclusion is based on low quality evidence, with questionable applicability to the Swiss context.

With respect to supplementation without prior testing, an examination of the literature suggests that the likelihood of adverse events occurring in patients who are not deficient but are given a supplement are low but include rare cases of anaphylaxis, as well as a possible increased risk of prostate cancer, colorectal adenomatous lesions and childhood asthma and wheezing.[55] Childhood asthma and wheezing is associated with maternal folic acid intake.[55] No natural history studies were identified to inform what would happen to deficient patients who do not receive a supplement (due to no testing or a false negative test result); however, reviews on folate deficiency indicate an association between untreated folate deficiency and megaloblastic anaemia, pancytopenia and increased homocysteine levels (associated with cardiovascular disease and stroke). The most recognised and studied adverse event related to folate deficiency is neural tube defect.[85]

Based on the limited evidence for the clinical effectiveness of folate testing, scenarios based on changes to reimbursement policy around folate testing could not be modelled. Assuming no change to the existing reimbursement arrangements, the budgetary impact of folate testing and supplementation is projected to increase from CHF 22.2 million in 2022 to CHF 30.8 million in 2026.

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14 Appendices

14.1 Appendix A: Literature sources (databases and websites)

Table 20 PubMed database search (inception to 1 August 2022): Search date 1 August 2022

#	Query	Results
1	Folic Acid[majr:noexp] OR Folic Acid Deficiency[majr:noexp] OR Homocysteine[majr:noexp] OR (folate*[ti] OR folic acid*[ti] OR folacin[ti] OR homocysteine[ti] OR methylenetetrahydrofol*[ti] OR tetrahydrofolate*[ti] OR tetrahydrofolic*[ti] OR vitamin B9[ti] OR folate*[ot] OR folic acid*[ot] OR homocysteine[ot] OR methylenetetrahydrofol*[ot] OR tetrahydrofolate*[ot] OR tetrahydrofolic*[ot] OR vitamin B9[ot])	39,372
2	Blood Chemical Analysis[majr:noexp] OR Diagnosis[majr:noexp] OR (analys*[ti] OR analyz*[ti] OR assay*[ti] OR biomarker*[ti] OR bio-marker*[ti] OR blood[ti] OR determin*[ti] OR diagnos*[ti] OR level[ti] OR levels[ti] OR measur*[ti] OR red cell[ti] OR red cells[ti] OR serum*[ti] OR status*[ti] OR test*[ti] OR analys*[ot] OR analyz*[ot] OR assay*[ot] OR biomarker*[ot] OR bio-marker*[ot] OR blood[ot] OR determin*[ot] OR diagnos*[ot] OR level[ot] OR levels[ot] OR measur*[ot] OR red cell[ot] OR red cells[ot] OR serum*[ot] OR status*[ot] OR test*[ot])	4,611,895
3	Folic Acid/blood[majr:noexp] OR Folic Acid Deficiency/blood[majr:noexp] OR Folic Acid Deficiency/diagnosis[majr:noexp] OR Homocysteine/blood[majr:noexp]	8,458
4	Dietary Supplements[mh:noexp] OR Folic Acid Deficiency[mh:noexp] OR Vitamins[mh:noexp] OR (deficien*[all] OR fortifi*[all] OR fortify*[all] OR supplement*[all] OR multivitamin*[ti] OR multivitamin*[ot] OR multi-vitamin*[ti] OR multi-vitamin*[ot] OR vitamin*[ti] OR vitamin[ot])	1,151,099
5	Diagnostic[tiab] OR diagnosis[tiab] OR sensitivity[tiab] OR specificity[tiab] OR positive predictive value[tiab] OR negative predictive value[tiab] OR accuracy[tiab]	3,594,366
6	"Economics"[Mesh:NoExp] OR "Costs and Cost Analysis"[mh] OR "Economics, Nursing"[mh] OR "Economics, Medical"[mh] OR "Economics, Pharmaceutical"[mh] OR "Economics, Hospital"[mh] OR "Economics, Dental"[mh] OR "Fees and Charges"[mh] OR "Budgets"[mh] OR budget*[tiab] OR economic*[tiab] OR cost[tiab] OR costs[tiab] OR costly[tiab] OR costing[tiab] OR price[tiab] OR prices[tiab] OR pricing[tiab] OR pharmacoeconomic*[tiab] OR "pharmaco-economic*"[tiab] OR expenditure[tiab] OR expenditures[tiab] OR expense[tiab] OR expenses[tiab] OR financial[tiab] OR finance[tiab] OR finances[tiab] OR financed[tiab] OR "value for money"[tiab] OR "monetary value*"[tiab] OR "models, economic"[mh] OR "economic model*"[tiab] OR "markov chains"[mh] OR markov[tiab] OR "monte carlo method"[mh] OR "monte carlo"[tiab] OR "Decision Theory"[mh] OR "decision tree*"[tiab] OR "decision analy*"[tiab] OR "decision model*"[tiab]†	1,369,128
7	"adverse effects"[subheading] OR complications[subheading] OR drug effects[subheading] OR safe[tw] OR safety[tw] OR side effect*[tw] OR undesirable effect*[tw] OR treatment emergent[tw] OR tolerability[tw] OR toxicity[tw] OR ADRs[tw] OR (adverse*[tw] AND (effect[tw] OR effects[tw] OR reaction[tw] OR reactions[tw] OR event[tw] OR events[tw] OR outcome[tw] OR outcomes[tw]))‡	8,169,568
8	"Clinical protocols"[MESH] OR "Consensus"[MESH] OR "Consensus development conferences as topic"[MESH] OR "Critical pathways"[MESH] OR "Guidelines as topic" OR "Practice guidelines as topic"[MESH] OR "Health planning guidelines"[MESH] OR "Clinical Decision Rules"[MESH] OR "guideline"[pt] OR "practice guideline"[pt] OR "consensus development conference"[pt] OR "consensus development conference, NIH"[pt] OR position statement*[tiab] OR policy statement*[tiab] OR practice parameter*[tiab] OR best practice*[tiab] OR standards[TI] OR guideline[TI] OR guidelines[TI] OR standards[ot] OR guideline[ot] OR guidelines[ot] OR guideline*[cn] OR standards[cn] OR consensus*[cn] OR recommendat*[cn] OR practice guideline*[tiab] OR treatment guideline*[tiab] OR CPG[tiab] OR CPGs[tiab] OR clinical guideline*[tiab] OR guideline recommendation*[tiab] OR consensus*[tiab] OR ((critical[tiab] OR clinical[tiab] OR practice[tiab]) AND (path[tiab] OR paths[tiab] OR pathway[tiab] OR pathways[tiab] OR protocol*[tiab] OR bulletin[tiab] OR bulletins[tiab])) OR recommendat*[ti] OR recommendat*[ot] OR (care[tiab] AND (standard[tiab] OR path[tiab] OR paths[tiab] OR pathway[tiab] OR pathways[tiab] OR map[tiab] OR maps[tiab] OR plan[tiab] OR plans[tiab])) OR (algorithm*[tiab] AND (screening[tiab] OR examination[tiab] OR test[tiab] OR tested[tiab] OR testing[tiab] OR assessment*[tiab] OR diagnosis[tiab] OR	1,465,446

	diagnoses[tiab] OR diagnosed[tiab] OR diagnosing[tiab])) OR (algorithm*[tiab] AND (pharmacotherap*[tiab] OR chemotherap*[tiab] OR chemotreatment*[tiab] OR therap*[tiab] OR treatment*[tiab] OR intervention*[tiab]))§	
9	(#1 AND #2) OR #3	13,667
10	#4 AND #9	6,098
11	"systematic"[filter] OR "meta-analysis"[pt] OR "meta-analysis as topic"[mh] OR "meta analy*"[tw] OR metanaly*[tw] OR metaanaly*[tw] OR "met analy*"[tw] OR "integrative research"[tiab] OR "integrative review*"[tiab] OR "integrative overview*"[tiab] OR "research integration*"[tiab] OR "research overview*"[tiab] OR "collaborative review*"[tiab] OR "collaborative overview*"[tiab] OR "systematic review"[pt] OR "systematic reviews as topic"[mh] OR "systematic review*"[tiab] OR "technology assessment*"[tiab] OR "technology overview*"[tiab] OR "technology appraisal*"[tiab] OR "Technology Assessment, Biomedical"[mh] OR HTA[tiab] OR HTAs[tiab] OR "comparative efficacy"[tiab] OR "comparative effectiveness"[tiab] OR "outcomes research"[tiab] OR "indirect comparison*"[tiab] OR "Bayesian comparison"[tiab] OR (("indirect treatment"[tiab] OR "mixed-treatment"[tiab]) AND comparison*[tiab]) OR Embase*[tiab] OR Cinahl*[tiab] OR "systematic overview*"[tiab] OR "methodological overview*"[tiab] OR "methodologic overview*"[tiab] OR "methodological review*"[tiab] OR "methodologic review*"[tiab] OR "quantitative review*"[tiab] OR "quantitative overview*"[tiab] OR "quantitative synthes*"[tiab] OR "pooled analy*"[tiab] OR Cochrane[tiab] OR Medline[tiab] OR Pubmed[tiab] OR Medlars[tiab] OR handsearch*[tiab] OR "hand search*"[tiab] OR "meta-regression*"[tiab] OR metaregression*[tiab] OR "data synthes*"[tiab] OR "data extraction"[tiab] OR "data abstraction*"[tiab] OR "mantel haenszel"[tiab] OR peto[tiab] OR "der-simonian"[tiab] OR dersimonian[tiab] OR "fixed effect*"[tiab] OR "multiple treatment comparison"[tiab] OR "mixed treatment meta-analys*"[tiab] OR "umbrella review*"[tiab] OR (("multiple paramet*"[tiab]) AND ("evidence synthesis"[tiab])) OR (("multi-paramet*"[tiab]) AND ("evidence synthesis"[tiab])) OR ((multiparameter*[tiab]) AND ("evidence synthesis"[tiab])) OR "Cochrane Database Syst Rev"[Journal] OR "health technology assessment winchester, england"[Journal] OR "Evid Rep Technol Assess (Full Rep)"[Journal] OR "Evid Rep Technol Assess (Summ)"[Journal] OR "Int J Technol Assess Health Care"[Journal] OR "GMS Health Technol Assess"[Journal] OR "Health Technol Assess (Rockv)"[Journal] OR "Health Technol Assess Rep"[Journal]‖	577,993
12	#10 AND #11	365
13	#5 AND #9	1,498
14	#6 AND #9	244
15	#7 AND #9	3,757
16	#8 AND #9	344
17	#12 OR #13 OR #14 OR #15 OR #16	5,429
18	Animals[MESH]	25,661,761
19	Humans[MESH]	20,632,022
20	#18 NOT (#18 AND #19)	5,023,739
21	Editorial[pt]	612,980
22	Letter[pt]	1,188,089
23	News[pt]	213,436
24	Congress[pt]	82,174
25	#21 OR #22 OR #23 OR #24	2,091,890
26	#17 NOT #20	5,013
27	#26 NOT #25	4,781

Notes

† Canadian Agency for Drugs and Technologies in Health (CADTH) search strings for economic evaluations & models[86]; ‡ Search string for adverse events by Su Golder[87]; § CADTH search strings for guidelines[88]; ‖ CADTH search strings for systematic reviews / meta-analyses / health technology assessments / indirect treatment comparisons[89]

Table 21 Embase database search (1974 to 2022 July 29): search date 1 August 2022

#	Query	Results
1	*Folic Acid/ OR *Folic Acid Deficiency/ OR *Homocysteine/ OR (folate*.ti. OR folic acid*.ti. OR folacin.ti. OR homocysteine.ti. OR methylenetetrahydrofol*.ti. OR tetrahydrofolate*.ti. OR tetrahydrofolic*.ti. OR vitamin B9.ti. OR folate*.kf. OR folic acid*.kf. OR homocysteine.kf. OR methylenetetrahydrofol*.kf. OR tetrahydrofolate*.kf. OR tetrahydrofolic*.kf. OR vitamin B9.kf.)	47,789
2	*Blood Chemistry/ OR *Diagnosis/ OR (analys*.ti. OR analyz*.ti. OR assay*.ti. OR biomarker*.ti. OR bio-marker*.ti. OR blood.ti. OR determin*.ti. OR diagnos*.ti. OR level.ti. OR levels.ti. OR measur*.ti. OR red cell.ti. OR red cells.ti. OR serum*.ti. OR status*.ti. OR test*.ti. OR analys*.kf. OR analyz*.kf. OR assay*.kf. OR biomarker*.kf. OR bio-marker*.kf. OR blood.kf. OR determin*.kf. OR diagnos*.kf. OR level.kf. OR levels.kf. OR measur*.kf. OR red cell.kf. OR red cells.kf. OR serum*.kf. OR status*.kf. OR test*.kf.)	5,425,044
3	*Folic Acid Deficiency/	2,640
4	*Dietary Supplement/ OR Folic Acid Deficiency/ OR Vitamin/ OR (deficien*.af. OR fortifi*.af. OR fortify*.af. OR supplement*.af. OR multivitamin*.ti. OR multivitamin*.kf. OR multi-vitamin*.ti. OR multi-vitamin*.kf. OR vitamin*.ti. OR vitamin.kf.)	3,132,452
5	Diagnostic.ti,ab,kf. OR diagnosis.ti,ab,kf. OR sensitivity.ti,ab,kf. OR specificity.ti,ab,kf. OR positive predictive value.ti,ab,kf. OR negative predictive value.ti,ab,kf. OR accuracy.ti,ab,kf.	4,799,762
6	Economics/ OR Cost/ OR exp Health Economics/ OR Budget/ OR budget*.ti,ab,kf. OR (economic* or cost or costs or costly or costing or price or prices or pricing or pharmacoeconomic* or pharmaco-economic* or expenditure or expenditures or expense or expenses or financial or finance or finances or financed).ti,kf. OR (economic* or cost or costs or costly or costing or price or prices or pricing or pharmacoeconomic* or pharmaco-economic* or expenditure or expenditures or expense or expenses or financial or finance or finances or financed).ab. /freq=2 OR (cost* adj2 (effective* or utilit* or benefit* or minimi* or analy* or outcome or outcomes)).ab,kf. OR (value adj2 (money or monetary)).ti,ab,kf. OR Statistical Model/ OR economic model*.ab,kf. OR Probability/ OR markov.ti,ab,kf. OR monte carlo method/ OR monte carlo.ti,ab,kf. OR Decision Theory/ OR Decision Tree/ OR (decision* adj2 (tree* or analy* or model*)).ti,ab,kf.†	1,876,744
7	"adverse effects".sh. OR complications.sh. OR drug effects.sh. OR safe.mp. OR safety.mp. OR side effect*.mp. OR undesirable effect*.mp. OR treatment emergent.mp. OR tolerability.mp. OR toxicity.mp. OR ADRs.mp. OR (adverse*.mp. AND (effect.mp. OR effects.mp. OR reaction.mp. OR reactions.mp. OR event.mp. OR events.mp. OR outcome.mp. OR outcomes.mp.))	4,669,044
8	exp clinical pathway/ OR exp clinical protocol/ OR clinical protocols/ OR exp consensus/ OR exp consensus development conference/ OR exp consensus development conferences as topic/ OR critical pathways/ OR exp guideline/ OR guidelines as topic/ OR exp practice guideline/ OR practice guidelines as topic/ OR health planning guidelines/ OR exp treatment guidelines/ OR Clinical Decision Rules/ OR (guideline or practice guideline or consensus development conference or consensus development conference, NIH).pt. OR (position statement* or policy statement* or practice parameter* or best practice*).ti,ab,kf. OR (standards or guideline or guidelines).ti,kf. OR ((practice or treatment* or clinical) adj guideline*).ab. OR (CPG or CPGs).ti. OR consensus*.ti,kf. OR consensus*.ab. /freq=2 OR ((critical or clinical or practice) adj2 (path or paths or pathway or pathways or protocol*)).ti,ab,kf. OR recommendat*.ti,kf. or guideline recommendation*.ab. OR (care adj2 (standard or path or paths or pathway or pathways or map or maps or plan or plans)).ti,ab,kf. OR (algorithm* adj2 (screening or examination or test or tested or testing or assessment* or diagnosis or diagnoses or diagnosed or diagnosing)).ti,ab,kf. OR (algorithm* adj2 (pharmacotherap* or chemotherap* or chemotreatment* or therap* or treatment* or intervention*)).ti,ab,kf. OR (guideline* or standards or consensus* or recommendat*).au. OR (guideline* or standards or consensus* or recommendat*).co. OR (guideline* or standards or consensus* or recommendat*).ca.‡	1,204,542
9	(#1 AND #2) OR #3	14,764
10	#4 AND #9	8,129
11	meta-analysis.pt. or meta-analysis/ or systematic review/ or meta-analysis as topic/ or "meta analysis (topic)"/ or "systematic review (topic)"/ or exp technology assessment, biomedical/ or ((systematic* adj3 (review* or overview*)) or (methodologic* adj3 (review* or overview*))).ti,ab,kw. or	876,613

	((quantitative adj3 (review* or overview* or synthes*)) or (research adj3 (integrati* or overview*))).ti,ab,kw. or ((integrative adj3 (review* or overview*)) or (collaborative adj3 (review* or overview*)) or (pool* adj3 analy*)).ti,ab,kw. or (data synthes* or data extraction* or data abstraction*).ti,ab,kw. or (handsearch* or hand search*).ti,ab,kw. or (mantel haenszel or peto or der simonian or dersimonian or fixed effect* or latin square*).ti,ab,kw. or (met analy* or metanaly* or technology assessment* or HTA or HTAs or technology overview* or technology appraisal*).ti,ab,kw. or (meta regression* or metaregression*).ti,ab,kw. or (meta-analy* or metaanaly* or systematic review* or biomedical technology assessment* or bio-medical technology assessment*).mp,hw. or (medline or ochrane or pubmed or medlars or embase or cinahl).ti,ab,hw. or (cochrane or (health adj2 technology assessment) or evidence report).jw. or (meta-analysis or systematic review).mp. or (comparative adj3 (efficacy or effectiveness)).ti,ab,kw. or (outcomes research or relative effectiveness).ti,ab,kw. or ((indirect or indirect treatment or mixed-treatment) adj comparison*).ti,ab,kw.§	
12	#10 AND #11	466
13	#5 AND #9	1,995
14	#6 AND #9	329
15	#7 AND #9	1,610
16	#8 AND #9	214
17	#12 OR #13 OR #14 OR #15 OR #16	4,081
18	Animal/	1,327,291
19	Human/	17,408,793
20	#18 NOT (#18 AND #19)	1,010,672
21	Editorial.pt.	732,437
22	Letter.pt.	1,233,045
23	Conference abstract.pt.	4,474,269
24	#21 OR #22 OR #23	6,439,751
25	#17 NOT #20	4,053
26	#25 NOT #24	3,446

Notes

† Canadian Agency for Drugs and Technologies in Health (CADTH) search string for economic evaluations & models[90]; ‡ CADTH search strings for guidleines[91]; § CADTH search strings for systematic reviews / meta-analyses / health technology assessments / indirect treatment comparisons[92]

Table 22 Natural History search string

#	Query	Results
1	(Folic Acid Deficiency[majr:noexp]) AND ((((((((((((cohort studies[mh]) OR (prognosis[mh])) OR (mortality[mh])) OR (morbidity[mh])) OR ("natural history")) OR (prognost*[tiab])) OR (course[tiab])) OR (predict*[tiab])) OR (outcome assessment[mh])) OR (outcome*[tiab])) OR (inception cohort*)) OR (disease progression[mh])) OR (survival analysis[mh]))	507

Table 23 Cochrane Library database search (inception to 26 July 2022): search date 26 July 2022

#	*	Results
1	MeSH descriptor: [Folic Acid] explode all trees] OR MeSH descriptor: [Folic Acid Deficiency] explode all trees OR MeSH descriptor: [Homocysteine] explode all trees OR (folate*:ti OR folic acid*:ti OR folacin:ti OR homocysteine:ti OR methylenetetrahydrofol*:ti OR tetrahydrofolate*:ti OR tetrahydrofolic*:ti OR vitamin B9:ti OR folate*:kw OR folic acid*:kw OR homocysteine:kw OR methylenetetrahydrofol*:kw OR tetrahydrofolate*:kw OR tetrahydrofolic*:kw OR vitamin B9:kw)	6,675
2	MeSH descriptor: [Blood Chemical Analysis] this term only OR MeSH descriptor: [Diagnosis] this term only OR (analys*:ti OR analyz*:ti OR assay*:ti OR biomarker*:ti OR bio-marker*:ti OR blood:ti OR determin*:ti OR diagnos*:ti OR level:ti OR levels:ti OR measur*:ti OR red cell:ti OR red cells:ti OR serum*:ti OR status*:ti OR test*:ti OR analys*:kw OR analyz*:kw OR assay*:kw OR biomarker*:kw OR bio-marker*:kw OR blood:kw OR determin*:kw OR diagnos*:kw OR level:kw OR levels:kw OR measur*:kw OR red cell:kw OR red cells:kw OR serum*:kw OR status*:kw OR test*:kw)	705,812
3	MeSH descriptor: [Folic Acid] explode all trees and with qualifier(s): [blood - BL] OR MeSH descriptor: [Folic Acid Deficiency] explode all trees and with qualifier(s): [blood - BL] OR MeSH descriptor: [Folic Acid Deficiency] explode all trees and with qualifier(s): [diagnosis – DI] OR MeSH descriptor: [Homocysteine] explode all trees and with qualifier(s): [blood - BL]	1,259
4	(#1 AND #2) OR #3	3,894
5	Limit to Cochrane Reviews	6

Table 24 Clinical trials search

#	Query	Date searched	Results
1	Folate AND (test OR testing OR diagnosis OR diagnostic)	6/9/2022	102

Table 25 Clinical practice guideline websites

Database	Website	Date searched	Results
Guidelines International Network (GIN)	https://www.g-i-n.net/library/international-guidelines-library	26/7/2022	Total hits: 0 Total included: 0
Association of Scientific Medical Societies (AWMF)	https://www.awmf.org/awmf-online-das-portal-der-wissenschaftlichen-medizin/awmf-aktuell.html	26/7/2022	Total hits: 4 Total included: 0
National Guideline Clearinghouse	https://www.ahrq.gov/gam/index.html	26/7/2022	Total hits: 2 Total included: 0
Scottish Intercollegiate Guidelines Network	https://www.sign.ac.uk/	26/7/2022	Total hits: 0 Total included: 0
Swiss Medical Weekly	https://smw.ch/en/	26/7/2022	Total hits: 2 Total included: 0
TRIP Database	https://www.tripdatabase.com/	27/7/2022	Total hits: 17 Total included: 4
Emergency Care Research Institute (ECRI) Guidelines Trust	https://www.ecri.org/solutions/ecri-guidelines-trust	27/7/2022	Unable to search

Table 26 Speciality society websites

Society	Website	Date searched	Results
Clinical Nutrition			
European Society for Clinical Nutrition and Metabolism (ESPEN) National Societies	https://www.espen.org/	9/5/2022	Total hits: 19 Total included: 0
Australasian Society for Parenteral and Enteral Nutrition	www.auspen.org.au	9/5/2022	Total hits: 0 Total included: 0
Flemish Society for Clinical Nutrition and Metabolism Vlaamse Vereniging voor Klinische Voeding en Metabolisme	www.vvkvm.be	9/5/2022	Unable to search
Société Belge de Nutrition Clinique Belgian Society of Clinical Nutrition	https://www.sbnc.site/	9/5/2022	Total hits: 0 Total included: 0
Canadian Nutrition Society Société Canadienne de Nutrition	www.cns-scn.ca	9/5/2022	Total hits: 0 Total included: 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. Purkyně	www.skvimp.cz	10/5/2022	Total hits: 0 Total included: 0
Dansk Selskab for Klinisk Ernæring Danish Society for Clinical Nutrition	www.dske.dk	10/5/2022	Total hits: 0 Total included: 0
The Finnish Society for Clinical Nutrition and Metabolism	www.fispen.fi	10/5/2022	Total hits: 1 Total included: 0
Société Francophone Nutrition Clinique et Métabolisme French Speaking Society of Clinical Nutrition and Metabolism	www.sfncm.org	10/5/2022	Total hits: 1 Total included: 0
Deutsche Gesellschaft für Ernährungsmedizin German Society for Nutritional Medicine	www.dgem.de	10/5/22	Total hits: 1 Total included: 0
Ελληνική Εταιρεία Ιατρικής/Κλινικής Διατροφής και Μεταβολισμού Hellenic Society for Clinical Nutrition and Metabolism	www.grespen.org	10/5/22	Total hits: 0 Total included: 0
Irish Society for Clinical Nutrition and Metabolism	www.irspen.ie	10/5/22	Total hits: 0 Total included: 0
Hachevra Letzona Clinit The Israeli Society for Clinical Nutrition	ISCN website	10/5/22	Total hits: 4 Total included: 0
Società Italiana di Nutrizione Artificiale e Metabolismo Italian Society for Artificial Nutrition and Metabolism	www.sinpe.org	10/5/22	Total hits: 2 Total included: 0
日本静脈経腸栄養学会 Japanese Society for Clinical Nutrition and Metabolism	https://www.jspen.or.jp/	10/5/22	Total hits: 0 Total included: 0
Norsk Selskap for Klinisk Ernæring Norwegian Society for Clinical Nutrition and Metabolism	www.nske.no	10/5/22	Total hits: 0 Total included: 0
Associação Portuguesa de Nutrição Entérica e Parentérica Portuguese Association of Enteral and Parenteral Nutrition	www.apnep.pt	10/5/22	Total hits: 0 Total included: 0

Society	Website	Date searched	Results
Society for Parenteral and Enteral Nutrition (Singapore)	http://www.singspen.com	10/5/22	Total hits: 0 Total included: 0
Slovensko Združenje za Klinično Prehrano Slovenian Society for Clinical Nutrition	http://klinicnaprehrana.si/	10/5/22	Total hits: 0 Total included: 0
Sociedad Española de Nutrición Clinica y Metabolismo Spanish Society of Clinical Nutrition and Metabolism	www.senpe.com	10/5/22	Total hits: 0 Total included: 0
The Swedish Society for Clinical Nutrition and Metabolism	www.swespen.se	10/5/22	Total hits: 0 Total included: 0
Gesellschaft für Klinische Ernährung der Schweiz/Société Suisse de Nutrition Clinique Swiss Society for Clinical Nutrition	www.geskes.ch	10/5/22	Total hits: 0 Total included: 0
Nederlandse Vereniging voor Gastro-Enterologie Netherlands Society for Parenteral and Enteral Nutrition	https://www.nvge.nl/	10/5/22	Total hits: 0 Total included: 0
British Association of Parenteral and Enteral Nutrition	www.bapen.org.uk	10/5/22	Total hits: 2 Total included: 0
American Society for Parenteral and Enteral Nutrition	www.nutritioncare.org	10/5/22	Total hits: 5 Total included: 0
Geriatric			
European Geriatric Medicine Society	https://www.eugms.org/home.html	10/5/22	Total hits: 0 Total included: 0
Australian and New Zealand Society for Geriatric Medicine	http://www.anzsgm.org/	10/5/22	Total hits: 1 Total included: 0
Schweizerische Fachgesellschaft für Geriatrie Swiss Geriatric Society	https://www.sfgg.ch/	10/5/22	Total hits: 0 Total included: 0
Haematology			
Belgian Hematology Society	http://www.bhs.be	10/5/22	Total hits: 0 Total included: 0
Cyprus Society of Haematology	www.cyhaema.com	10/5/22	Total hits: 0 Total included: 0
Česká Hematologická Společnost CLS JEP Czech Society of Hemtology	http://www.hematology.cz	10/5/22	Total hits: 0 Total included: 0
Dansk Haematologisk Selskab Danish Society of Hematology	http://www.hematology.dk	10/5/22	Total hits: 0 Total included: 0
Suomen hematologiyhdistys Finnish hematology Association	www.hematology.fi/en	10/5/22	Total hits: 0 Total included: 0
Société Française d'Hématologie French Society of Hematology	http://sfh.hematologie.net	10/5/22	Total hits: 0 Total included: 0
Deutsche Gesellschaft für Hämatologie und Medizinische Onkologie German Society of Hematology	http://www.dgho.de	10/5/22	Total hits: 0 Total included: 0
Eliniki Ematologhiki Eteria Hellenic Society of Hematology	http://www.eae.gr	10/5/22	Total hits: 0 Total included: 0
Haematology Association of Ireland	http://www.haematologyireland.org	10/5/22	Total hits: 0 Total included: 0
Israel Society of Hematology and Transfusion Medicine	http://www.hematology.org.il	10/5/22	Unable to search

Society	Website	Date searched	Results
Società Italiana di Ematologia Italian Society of Hematology	http://www.siematologia.it	9/5/22	Total hits: 0 Total included: 0
Société Luxembourgoise d'Oncologie	https://www.slo.lu	9/5/22	Total hits: 0 Total included: 0
Sociedade Portuguesa de Hematologia Portugese Society of Hematology	http://www.sph.org.pt	9/5/22	Total hits: 0 Total included: 0
Zdruzenja Hematologov Slovenije Slovenian Society of Hematology	http://www.hematologija.org	9/5/22	Total hits: 0 Total included: 0
Sociedad Española de Hematología y Hemoterapia	http://sehh.es/es/	9/5/22	Total hits: 0 Total included: 0
Svensk Förening för Hematologi Swedish Society of Hematology	http://www.sfhem.se	9/5/22	Total hits: 0 Total included: 0
Schweizerische Gesellschaft für Hämatologie Swiss Society for Hematology	http://www.sgh-ssh.ch	9/5/22	Total hits: 0 Total included: 0
Nederlandse Vereniging voor Hematologie Dutch Society of Hematology	https://www.hematologienederland.nl	9/5/22	Total hits: 0 Total included: 0
British Society for Haematology	http://www.b-s-h.org.uk	9/5/22	Total hits: 1 Total included: 0
Neurology			
Brain Research Society of Finland	https://www.brsf.org/	9/5/22	Total hits: 0 Total included: 0
British Neuroscience Association	https://www.bna.org.uk/	9/5/22	Total hits: 0 Total included: 0
Hrvatski Institut za Istraživanje Mozga Croatian Society for Neuroscience	http://www.hiim.unizg.hr/	9/5/22	Total hits: 0 Total included: 0
Czech Neuroscience Society	https://www.biomed.cas.cz/cns/index.php/en/	9/5/22	Total hits: 0 Total included: 0
Dansk Selskab for Neurovidenskab Danish Society for Neuroscience	https://dsfn.dk/	9/5/22	Total hits: 0 Total included: 0
Dutch Neurofederation	https://neurofederatie.nl/	9/5/22	Total hits: 0 Total included: 0
German Neuroscience Society	https://nwg-info.de/	9/5/22	Total hits: 0 Total included: 0
Hellenic Society for Neuroscience	https://www.hsfn.gr/	9/5/22	Total hits: 0 Total included: 0
Israel Society for Neuroscience	https://www.isfn.org.il/	9/5/22	Total hits: 0 Total included: 0
Società Italiana di Neuroscienze Italian Society for Neuroscience	http://www.sins.it/EN/index.xhtml	9/5/22	Total hits: 0 Total included: 0
Malta Neuroscience Network	http://mnn.mt/	9/5/22	Total hits: 0 Total included: 0
Neuroscience Ireland	https://neuroscienceireland.com/	9/5/22	Total hits: 0 Total included: 0
Norwegian Neuroscience Society	https://www.ntnu.edu/nns	9/5/22	Total hits: 0 Total included: 0
Slovenian Neuroscience Association	http://www.sinapsa.org/naslovnica/	9/5/22	Total hits: 0 Total included: 0

Society	Website	Date searched	Results
Sociedad Española de Neurociencia Spanish Society of Neuroscience	https://www.senc.es/en/	9/5/22	Total hits: 0 Total included: 0
Sociedade Portuguesa de Neurociências Portuguese Society for Neuroscience	http://www.spn.org.pt/	9/5/22	Total hits: 0 Total included: 0
Societé des Neurosciences The French Neuroscience Society	https://www.neurosciences.asso.fr/	9/5/22	Total hits: 0 Total included: 0
Swiss Society for Neuroscience	https://www.swissneuroscience.ch/	9/5/22	Total hits: 0 Total included: 0

Table 27 HTA websites

HTA agency	Source	Date searched	Results
International			
International HealthTechScan	https://www.i-hts.org/	10/5/22	Unable to search
International Network of Agencies for Health Technology Assessment (INAHTA)	https://database.inahta.org/	10/5/22	Total hits: 9 Total included: 0
Adelaide Health Technology Assessment (AHTA)	https://www.adelaide.edu.au/ahta/pubs/	10/5/22	Total hits: 0 Total included: 0
Australian Safety and Efficacy Register of New Interventional Procedures – Surgical (ASERNIP–S)	https://www.surgeons.org/research-audit/research-evaluation-inc-asernips	10/5/22	Total hits: 0 Total included: 0
Austria			
Austrian Institute for Health Technology Assessment (AIHTA)	https://aihta.at/page/homepage/en	25/7/22	Total hits: 1 Total included: 0
Gesundheit Österreich GmbH (GOG)	http://www.goeg.at	25/7/22	Total hits: 0 Total included: 0
Argentina			
Institute for Clinical Effectiveness and Health Policy (IECS)	http://www.iecs.org.ar	25/7/22	Total hits: 0 Total included: 0
Belgium			
Belgian Health Care Knowledge Centre (KCE)	http://kce.fgov.be	25/7/22	Total hits: 0 Total included: 0
Brazil			
National Committee for Technology Incorporation (CONITEC)	http://conitec.gov.br/en/	25/7/22	Total hits: 1 Total included: 0
Agência Nacional de Saúde Suplementar/ National Regulatory Agency for Private Health Insurance and Plans (ANS)	https://www.gov.br/ans/pt-br	25/7/22	Total hits: 0 Total included: 0
Canada			
Institute of Health Economics (IHE)	http://www.ihe.ca	10/5/22	Total hits: 0 Total included: 0
Institut National d'Excellence en Santé et en Services Sociaux (INESSS)	https://www.inesss.qc.ca/en/home.html	10/5/22 Screened first 5 pages of results	Total hits: 3 Total included: 1
The Canadian Agency for Drugs and Technologies in Health (CADTH)	http://www.cadth.ca/	10/5/22	Total hits: 4 Total included: 2
Ontario Health (OH)	https://www.ontariohealth.ca/	25/7/22	Total hits: 0 Total included: 0

HTA agency	Source	Date searched	Results
Denmark			
Social & Health Services and Labour Market (DEFACTUM)	http://www.defactum.net	10/5/22	Total hits: 0 Total included: 0
Finland			
Finnish Coordinating Center for Health Technology Assessment (FinCCHTA)	https://www.ppshp.fi/Tutkimus-ja-opetus/FinCCHTA/Sivut/default.aspx	10/5/22	Total hits: 0 Total included: 0
Finnish Medicines Agency (FIMEA)	http://www.fimea.fi	10/5/22	Total hits: 1 Total included: 0
France			
Haute Autorité de Santé (HAS) French National Authority for Health	http://www.has-sante.fr/	10/5/22	Total hits: 1 Total included: 0
Comité d'Evaluation et de Diffusion des Innovations Technologiques (CEDIT)	http://cedit.aphp.fr/	10/5/22	Total hits: 0 Total included: 0
Germany			
Institute for Quality and Efficiency in Health Care (IQWiG)	http://www.iqwig.de	10/5/22	Total hits: 0 Total included: 0
Gemeinsamer Bundesausschuss (G-BA) Federal Joint Committee	https://www.g-ba.de/english/	25/7/22	Total hits: 0 Total included: 0
Ireland			
Health Information and Quality Authority (HIQA)	http://www.hiqa.ie	10/5/22	Total hits: 0 Total included: 0
Italy			
Agenzia Sanitaria e Sociale Regionale (ASSR)	https://assr.regione.emilia-romagna.it/	10/5/22	Total hits: 0 Total included: 0
HTA Unit in A. Gemelli Teaching Hospital (UVT)	https://www.policlinicogemelli.it/	10/5/22	Total hits: 0 Total included: 0
National Agency for Regional Health services (Agenas)	http://www.agenas.it	11/5/22	Total hits: 0 Total included: 0
Kazakhstan			
Ministry of Public Health of the Republic of Kazakhstan, Republican Centre for Health Development (RCHD)	http://www.rcrz.kz	25/7/22	Total hits: 0 Total included: 0
Korea			
National Evidence-based healthcare Collaborating Agency (NECA)	http://www.neca.re.kr/eng www.neca.re.kr/eng	25/7/22	Unable to search
Malayasia			
Health Technology Assessment Section, Ministry of Health Malaysia (MaHTAS)	http://www.moh.gov.my	25/7/22	Total hits: 0 Total included: 0
the Netherlands			
the Netherlands Organisation for Health Research and Development (ZonMw)	http://www.zonmw.nl	25/7/22	Total hits: 3 Total included:
Zorginstituut Nederland (ZIN)	https://www.zorginstituutnederland.nl/	11/5/22	Total hits: 0 Total included: 0
Norway			
The Norwegian Institute of Public Health (NIPH)	http://www.fhi.no/	11/5/22	Total hits: 18 Total included: 0
Peru			
Institute of Health Technology Assessment and Research (IETSI)	http://www.essalud.gob.pe/ietsi/	25/7/22	Total hits: 0 Total included: 0

HTA agency	Source	Date searched	Results
Poland			
Agency for Health Technology Assessment and Tariff System (AOTMiT)	http://www.aotm.gov.pl	25/7/22	Total hits: 0 Total included: 0
Republic of China, Taiwan			
Center for Drug Evaluation (CDE)	http://www.cde.org.tw	25/7/22	Total hits: 0 Total included: 0
Russian Federation			
Center for Healthcare Quality Assessment and Control (CHQAC)	www.rosmedex.ru	25/7/22	Total hits: 0 Total included: 0
Singapore			
Agency for Care Effectiveness (ACE)	http://www.ace-hta.gov.sg/	11/5/22	Total hits: 0 Total included: 0
Spain			
Agencia de Evaluación de Tecnologias Sanitarias, Instituto de Salud Carlos III Health Technology Assessment Agency (AETS)	http://publicaciones.isciii.es/	25/7/22	Total hits: 0 Total included: 0
Agency for Health Quality and Assessment of Catalonia (AQuAS)	http://aquas.gencat.cat	25/7/22	Total hits: 0 Total included: 0
Andalusian HTA Agency (AETSA)	http://www.aetsa.org/	25/7/22	Total hits: 0 Total included: 0
Basque Office for Health Technology Assessment (OSTEBA)	https://www.euskadi.eus/web01-a2ikeost/en/	25/7/22	Total hits: 0 Total included: 0
Galician Agency for Health Technology Assessment (AVALIA-T)	http://acis.sergas.es	26/7/22	Total hits: 0 Total included: 0
Health Sciences Institute in Aragon (IACS)	http://www.iacs.es/	26/7/22	Total hits: 0 Total included: 0
Sweden			
Swedish Agency for Health Technology Assessment and Assessment of Social Services (SBU)	http://www.sbu.se/en/	26/7/22	Total hits: 1 Total included: 0
Switzerland			
Swiss Federal Office of Public Health (SFOPH)	http://www.bag.admin.ch/hta	26/7/22	Total hits: 0 Total included: 0
Tunisia			
INEAS – National Authority for Assessment and Accreditation in Healthcare, TUNISIA	Https://www.inease.tn	25/7/22	Total hits: 0 Total included: 0
United Kingdom			
Healthcare Improvement Scotland (HIS)	http://www.healthcareimprovementscotland.org	25/7/22	Total hits: 0 Total included: 0
National Institute for Health and Care Excellence (NICE)	http://www.nice.org.uk/	25/7/22	Total hits: 4 Total included: 0
Health Technology Wales (HTW)	http://www.healthtechnology.wales	25/7/22	Total hits: 0 Total included: 0
National Institute for Health Research (NIHR), including HTA programme	https://evidence.nihr.ac.uk/	25/7/22	Total hits: 0 Total included: 0
United States			
Agency for Healthcare Research and Quality (AHRQ)	https://www.ahrq.gov/research/findings/index.html	5/11/22	Total hits: 2 Total included: 0
Uruguay			
Health Assessment Division, Ministry of Public Health (HAD)	http://www.msp.gub.uy	25/7/22	Total hits: 0 Total included: 0

14.2 Appendix B: Evidence tables

Table 28 Study characteristics (diagnostic accuracy)

Study ID; Setting; Country	Study design Follow-up n	Inclusion criteria	Patient characteristics	Test characteristics	Reference standard	Folate deficiency threshold	Findings
Bain et al. (1984)[46]; Tertiary hospital; UK	Diagnostic cohort study NR n=110	All laboratory samples sent for RBC folate between Jan 1981–July 1983, plus 50 haematologically normal inpatients	**Group 1 Definite folate deficiency (n=18):** Megaloblastic bone marrow, abnormal deoxyuridine suppression test, normal B12, normal B12 absorption **Group 2 Probably folate deficiency (n=7):** As above, B12 not measured **Group 3 Definitely B12 deficient with no reason to suspect folate deficiency (n=35):** Megaloblastic bone marrow, abnormal deoxyuridine suppression test, low serum B12, B12 malabsorption (or known total gastrectomy) **Group 4 Probably B12 deficient with no reason to suspect folate deficiency (n=3):**	RBC folate assay: competitive protein binding assay (Bettcon Dickinson Radioassay Kit, derived from milk)	Patients were investigated for folate and B12 deficiency irrespective of RBC test using bone marrow aspirate, deoxyuridine suppression test, dietary intake assessment, small bowel biopsy and other investigations for malabsorption.	200 microgram per L (derived from 95% confidence limits in 200 normal subjects)	**Sensitivity** (Group 1 and 2 combined): 96% (TP = 24, FN = 1) **Specificity** (Group 3, 4 and 5 combined): 71% (TN = 59, FP = 24)

			As above, but Schilling test not done **Group 5 Macrocyclic but not folate deficient (n=45):** deoxyuridine suppression test normal **Group 6 Possible deficiency of both B12 and folic acid (n=2), i.e. Equivocal results**: Coeliac disease with low serum B12 or abnormal B12 absorption .				

Abbreviations

FN = false negative; **FP** = false positive; **NR** = not reported; **RBC** = red blood cell; **TN** = true negative; **TP** = true positive; **UK** = United Kingdom.

Table 29 Study characteristics (management decisions following folate testing studies)

Study ID; Setting; Country	Study design Follow-up n	Inclusion criteria	Patient characteristics	Test characteristics	Folate deficiency threshold
Jaffe and Schilling (1990)[49]; Tertiary hospital; USA	Retrospective case series NR n = 1,355	Inpatients with serum and RBC folate levels measured	RBC folate ≤175 ng/ml (≤397nmol/L) Age: 49.6 years ± 17.6[a] Male:74% Serum folate: 4.4 ng/ml ± 4.4 (10nmol/L±10)[a] Serum B12: 577.5 ng/ml ± 664.6 (1308.6nmol/L±1506.0)[a]	Solid Phase No Boil Dualcount Radioassay (Diagnostic Products Corporation, Los Angeles, CA)	RBC: 175 ng/ml (397 nmol/L)
Vinker et al. (2013)[54]; Metropolitan primary care clinics (13 in total); Israel	Retrospective case series NR n = 152	All folate determinations requested for patients 17–75 years with low serum folate (<5.6 nmol/L [<2.5ng/ml]) in 13 urban clinics in Israel between 1 Jan 2004 and 1 Jan 2007. Excluded: Patients who required folic acid monitoring or who were at risk of folate deficiency (e.g. malabsorption, schizophrenia, residing in old age home or other institution, pregnant women, haemodialysis patients and those treated with antiepileptics or methotrexate)	Age: 48.2 years ± 19[a] % male: 45.4%	NR	Serum folate <5.6 nmol/L (<2.5ng/ml)
Singh et al. (2015)[52]; Tertiary hospitals (2 centres); USA	Retrospective case series NR n = 253 (226 patients analysed)	All folate determinations at 2 centres between June 2013 and June 2014 were reviewed. Patients with serum folate <5.5 ng/ml (<12.5nmol/L) were retrieved for chart review.	Age: 53 years ± 14.9[a] BMI: 28.5 ± 7.85	NR	Serum folate <3.4 ng/ml (<7.7 nmol/L) (deficient) <5.5 ng/ml (<12.5 nmol/L) (insufficient)
Robinson and Mladenovic (2001)[51]; Inpatient and outpatient hospital (3 centres);	Retrospective case series NR	All records of inpatients with a folate test result over 1 year	NR	NR	Serum folate defined as from <1.5 ng/ml (<3.4 nmol/L) to <2.8 ng/ml (<6.3 nmol/L)

USA	n = 2,998				among the 3 centres.
Theisen-Toupal et al. (2013)[53]; Tertiary hospital; USA	Retrospective case series NR n = 2,093	All serum folate tests ordered in inpatient units and emergency department between 1 Jan 2011 and 31 Dec 2011.	Age: 66 years[b] Male: 50.8%	Serum folate: chemiluminescent competitive binding protein assay on E170 analyser (Roche diagnostics, Indianapolis, IN)	Serum folate Deficient: ≤3.0 ng/ml (≤6.8 nmol/L) Low normal: 3.0–3.9 ng/ml (6.8–8.8 nmol/L)
Latif et al. (2004)[50]; Inpatient and outpatient hospital; USA	Retrospective case series NR n = 6,024 samples from 4,985 patients	All serum and RBC tests performed on inpatient and outpatient patients in 2001. Charts from patients with low serum folate were reviewed	Low serum folate Age: 59 years (19–93)[c] Normal serum folate Age: 67 years (3–87)[c]	Commercially available assay (Folate, Elecsys 2010 system)	Serum folate <2.8 ng/ml (<6.3 nmol/L) RBC folate <175 ng/ml (<397 nmol/L)
Ashraf et al. (2008)[47]; Inpatient and outpatient hospital; USA	Retrospective case series NR n = 1,007 samples from 980 patients	All serum folate levels obtained during a 4-month period. Charts were review for all patients with low or borderline folate levels.	Age: 63.8 years[d] Male: 38% Indication: anaemic (60%)	NR	Serum folate Low: <3.0 ng/ml (<6.8 nmol/L) Borderline: 3–4 ng/ml (6.8–9.1 nmol/L)
Bor et al. (2008)[48]; Single laboratory with inpatient and outpatient requests, Denmark	Retrospective case series NR n = 12,932 patients	All RBC folate analyses requested by hospital doctors (26%) or general practitioners (74%) in 2003. Charts were reviewed for all patients referred from the hospital with an abnormal folate result	Hospital patients: Age male: 56 ± 19 years[a] Age female: 54 years ± 20 years[a] Male: 39%		RBC folate <350 nmol/L (<155 ng/ml)

Abbreviations

CA = California; **IN** = Indiana; **NR** = not reported; **RBC** = red blood cell; **USA** = United States of America.

Notes

a = mean ± SD; **b** = median; **c** = median (range); **d** = mean.

Table 30 Study findings (management decisions following folate testing studies)

Study ID; Setting; Country	Findings
Jaffe and Schilling (1990)[49]; Tertiary hospital; USA	- 62/1355 patients (4.6%) had RBC folate ≤ 175 ng/ml (≤397 nmol/L) (57 with medical records adequate for review) - 20/57 (35%) patients with low folate were treated with supplementation - 11/20 (55%) patients responded to supplementation (increase in haemoglobin of 1% or reduction in MCV to normal) - 13 patients treated with supplementation had both low RBC and serum folate levels, 8/13 responded to treatment - 7 patients treated with supplementation had low RBC but normal serum folate levels, 3/7 responded to treatment
Vinker et al. (2013)[54]; Metropolitan clinics (13 clinics); Israel	- 4.3% of all folate tests (43,176) were positive for folate deficiency - 68/152 (44.7%) of patients with low folate (who met the inclusion criteria for this study) were treated with supplementation - 1.9% of folate tests therefore resulted in a change in management
Singh et al. (2015)[52]; Tertiary hospitals (2 centres); USA	- Of all folate tests ordered, 4.9% identified patients with folate deficiency or insufficiency - 88/226 (38.9%) patients with folate deficiency or insufficiency were treated with supplementation
Robinson and Mladenovic (2001)[51]; Inpatient (3 centres); USA	- 68/2998 (2.3%) of folate measurements were low - 35/68 (53%) of low folate levels were recorded - 17/68 (24%) of low folate levels were attributed to a possible cause - 16/68 (24%) of low folate levels were treated with supplementation - Overall clinical response to folate testing was 0.5% - Recommend that folic acid levels are rarely low in patients with macrocytosis and low levels have little influence on therapy - Recommend only check cobalamin levels and, if normal, treat empirically with folic acid - Recommend folate tests for patients with persistent unexplained macrocytic anaemia
Theisen-Toupal et al. (2013)[53]; Tertiary hospital; USA	- 2/2093 (0.1%) folates tests were deficient - 7/2093 (0.3%) were low-normal - 1/2 (50%) patients with folate deficiency were treated with supplementation
Latif et al. (2004)[50]; Inpatient and outpatient; USA	- 77/4689 (1.6%) serum folate tests were low - 16/1335 (1.2%) RBC folate tests were low - Overall, 74/4315 patients had low folate levels - 39/63 (62%) patients with low folate received supplementation (only 63 patients had their folate results available for review) - 39/4315 (0.9%) of patients clinically suspected of folate deficiency received supplementation - Indication for folate testing did not appear to influence physician response to test results

Ashraf et al. (2008)[47]; Inpatient and outpatient hospital; USA	- 4/980 (0.4%) patients had low folate levels - 10/980 (1.0%) patients had borderline folate levels - 5/14 (36%) patients with low/borderline folate received supplementation
Bor et al. (2008)[48]; Single laboratory with inpatient and outpatient requests; Denmark	- RBC folate levels were low in 1.7% of tests requested by hospital doctors and 1.3% of tests requested by General Practitioners - Low RBC folate was observed more often in women <40 years old - 31/57 (54%) of hospital patients with low folate levels were prescribed folic acid - 26/57 (46%) of low folate tests requested by hospital doctors were noted in the medical records - Overall clinician response to folate testing was 34/57 (60%) - Chart review was not possible for folate tests requested by general practitioners

Abbreviations
MCV = mean corpuscular volume; **RBC** = red blood cell; **USA** = United States of America.

14.3 Appendix C: Quality appraisal of included studies

Table 31 QUADAS-2 risk of bias assessment[42]

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)
Bain et al. (1984)[46]	?	😊	?	☹	?	☹	?

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

Table 32 IHE risk of bias assessment

IHE Domain	Jaffe and Schilling (1990)[49]	Vinker et al. (2013)[54]	Singh et al. (2015)[52]	Robinson and Mladenovic (2001)[51]	Theisen-Toupal et al. (2013)[53]	Latif et al. (2004)[50]	Ashraf et al. (2008)[47]	Bor et al. (2008)[48]
Study objective								
Objective clearly stated	Y	Y	Y	Y	Y	Y	Y	Y
Study design								
Prospective	N	N	N	N	N	N	N	N
Multicentre	N	Y	Y	Y	N	N	N	Y
Consecutive recruitment	Y	Y	Y	Y	Y	Y	Y	Y
Study population								
Were patient characteristics included?	P	P	P	P	P	P	Y	P
Eligibility criteria clearly stated	Y	Y	Y	Y	Y	Y	Y	Y
Did patient enter the study at a similar point in the disease	NA	NA	NA	NA	NA	NA	NA	NA
Intervention and co-intervention								
Was the method of intervention clearly described? [a]	Y	P	P	P	Y	Y	P	P
Were additional interventions clearly described?	NA	NA	NA	NA	NA	NA	NA	NA
Outcome measure								
Were relevant outcome measures established *a priori*?	Y	Y	Y	Y	Y	Y	Y	Y
Were outcome assessors blinded to the intervention?	NA	NA	NA	NA	NA	NA	NA	NA
Were the outcomes measured using appropriate objective methods?	Y	Y	Y	Y	Y	Y	Y	Y

IHE Domain	Jaffe and Schilling (1990)[49]	Vinker et al. (2013)[54]	Singh et al. (2015)[52]	Robinson and Mladenovic (2001)[51]	Theisen-Toupal et al. (2013)[53]	Latif et al. (2004)[50]	Ashraf et al. (2008)[47]	Bor et al. (2008)[48]
Were the relevant outcome measures made before and after the intervention?	NA	NA	NA	NA	NA	NA	NA	NA
Statistical analysis								
Were the statistical tests used to assess the relevant outcomes appropriate?	NA	NA	NA	NA	NA	NA	NA	NA
Results and conclusions								
Was follow-up long enough for important events and outcomes to occur?	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear
Were losses to follow-up reported?	NA	NA	NA	NA	NA	NA	NA	NA
Did study provide estimates of random variability in the data analysis of relevant outcomes?	NA	NA	NA	NA	NA	NA	NA	NA
Were the adverse events reported?	NA	NA	NA	NA	NA	NA	NA	NA
Were the conclusions supported by results?	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?	N	Y	P	N	P	N	Y	N

Abbreviations
N = No; **NA** = not applicable; **P** = Partial ; **Y** = Yes.

Table 33 AMSTAR 2 appraisal of systematic review (applied to an umbrella review)

AMSTAR 2 domain	Bo (2020)[55]
1. Did the research questions and inclusion criteria for the review include the components of PICO?	Y
2. Did the report of the review contain an explicit statement that the review methods were established prior to the conduct of the review and did the report justify any significant deviations from the protocol?	N
3. Did the review authors explain their selection of the study designs for inclusion in the review?	Y
4. Did the review authors use a comprehensive literature search strategy?	P[a]
5. Did the review authors perform study selection in duplicate?	Y
6. Did the review authors perform data extraction in duplicate?	Y
7. Did the review authors provide a list of excluded studies and justify the exclusions?	N
8. Did the review authors describe the included studies in adequate detail?	P
9. Did the review authors use a satisfactory technique for assessing the risk of bias (RoB) in individual studies that were included in the review?	Y[b]
10. Did the review authors report on the sources of funding for the studies included in the review?	P[c]
11. If meta-analysis was performed did the review authors use appropriate methods for statistical combination of results?	Y
12. If meta-analysis was performed, did the review authors assess the potential impact of RoB in individual studies on the results of the meta-analysis or other evidence synthesis?	Y
13. Did the review authors account for RoB in individual studies when interpreting/ discussing the results of the review?	Y
14. Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review?	Y
15. If they performed quantitative synthesis did the review authors carry out an adequate investigation of publication bias (small study bias) and discuss its likely impact on the results of the review?	N
16. Did the review authors report any potential sources of conflict of interest, including any funding they received for conducting the review?	Y

Abbreviations

N = No; **NA** = not applicable; **P** = Partial ; **PICO** = population, intervention, comparator, outcomes ; **RoB** = risk of bias; **Y** = Yes.

Notes

a = The review did not search grey literature sources, **b** = Risk of bias was appropriately conducted for meta-analyses using the AMSTAR-2 tool, **c** = the review considered sources of funding for the included studies when conducting the AMSTAR 2 appraisal. These were not reported separately; noting most included reviews did not report these.