Indicators for assessing folate and vitamin B-12 status and for monitoring the efficacy of intervention strategies[1–3]

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ABSTRACT

Deficiencies of folate or of vitamin B-12 are widespread and constitute a major global burden of morbidity that affect all age groups. Detecting or confirming the presence of folate or vitamin B-12 deficiency and distinguishing one from the other depends, ultimately, on laboratory testing. Tests to determine the presence of folate or vitamin B-12 deficiency are used singly or in combination to establish the nutritional status and prevalence of deficiencies of the vitamins in various populations. The efficacy of interventions through the use of fortification or supplements is monitored by using the same laboratory tests. Tests currently in use have limitations that can be either technical or have a biological basis. Consequently, each single test cannot attain perfect sensitivity, specificity, or predictive value. Laboratory indicators of vitamin B-12 or folate status involve the measurement of either the total or a physiologically relevant fraction of the vitamin in a compartment such as blood. Thus, assays to measure vitamin B-12 or folate in plasma or serum as well as folate in red blood cells are in widespread use, and more recently, methods to measure vitamin B-12 associated with the plasma binding protein transcobalamin (holotranscobalamin) have been developed. Alternatively, concentrations of surrogate biochemical markers that reflect the metabolic function of the vitamin can be used. Surrogates most commonly used are plasma homocysteine, for detection of either vitamin B-12 or folate deficiency, and methylmalonic acid for detection of vitamin B-12 deficiency. The general methods as well as their uses, indications, and limitations are presented. *Am J Clin Nutr* 2011;94(suppl):666S–72S.

INTRODUCTION

Although terminologically inconsistent, common usage has favored the terms *folate* and *vitamin B-12* for the 2 vitamins that form the subject of this communication. Preferred biochemical nomenclature for vitamin B-12 is cobalamin, which is the generic term for the group of cobamide vitamers that all possess the base 5,6-dimethylbenzimidazole as the lower axial ligand coordinated to the central cobalt atom and one of several upper axial ligands (CN, OH, methyl, adenosyl, and others). The term *vitamin B12* referred originally to the form of cobalamin that was first isolated and characterized, which was cyanocobalamin. The designation of folate in the B-vitamin numbering system is B9, but this term is rarely used.

For 2 related reasons, the nutrients folate (vitamin B-9) and vitamin B-12 (B12, cobalamin) are inextricably linked. Metabolically, both vitamins participate in an enzyme reaction involving one-carbon metabolism in which the substrate homocysteine is converted to methionine through transfer of a methyl group from the cosubstrate methyltetrahydrofolate. This enzyme, methionine synthase, requires vitamin B-12 in the form of methylcobalamin as a cofactor, and it is at the crossroads linking the important pathway of methylation through synthesis of *S*-adenosyl methionine and the pathways of purine and pyrimidine (thymidine) synthesis through generation of tetrahydrofolate. Deficiency of either vitamin can result in perturbation of these 2 key pathways with consequent disruption of DNA synthesis caused by thymidine lack and resulting megaloblastic anemia. In addition, deficiency of these vitamins can result in disturbances of methylation, leading to effects on the nervous system and other organs. Because of the adverse consequences of such deficiencies, and the potential to prevent and correct them, the ability to assess folate and vitamin B-12 status is important for public health. The hematologic complications of folate or vitamin B-12 deficiencies are identical so that for effective treatment it is critical to have a reliable means to discriminate between these 2 vitamin deficiencies. The ability to detect or confirm the presence of folate or vitamin B-12 deficiency and to distinguish one from the other rests on dependable laboratory testing. Another aspect of the importance of laboratory testing relates to the importance of monitoring the efficacy of intervention programs designed to prevent or ameliorate deficiencies of folate and vitamin B-12.

The methods used to assess folate and vitamin B-12 status fall broadly into 1 of 2 categories. In one, concentrations of the vitamins are measured directly in the blood and in the other, metabolites that accumulate as a result of the vitamin deficiencies are measured. Tests to determine the presence of folate or vitamin B-12 deficiency may be used singly or in combination to establish the nutritional status and prevalence of deficiencies of the vitamins in various populations. Deficiencies of folate or vitamin B-12 ultimately cause structural or morphologic changes that

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most conspicuously become evident through changes in the blood, including anemia with associated morphologic changes.

In essence, tests to assess folate or vitamin B-12 status can also be used as surrogates for monitoring the efficacy of intervention. However, correction of an abnormal laboratory value does not necessarily connote a successful outcome of the intervention unless there is evidence of objective improvement in the health of an individual or there is a reduction of disease prevalence in a population. Mere "improvement" of a laboratory value toward normality does not constitute a priori evidence of benefit. Before discussing the indicators for assessing folate and vitamin B-12 status, the causes of deficiency of these vitamins will be reviewed briefly.

CAUSES OF FOLATE AND VITAMIN B-12 DEFICIENCIES

There are numerous known causes of deficiency of folate or vitamin B-12. In some situations, such as chronic generalized malabsorption or malnutrition, deficiencies of both vitamins may coexist. A detailed consideration of all causes of folate or vitamin B-12 deficiency lies beyond the scope of this communication, and for this, the reader is referred to several recent reviews of the subject (1–3). Briefly, causes of deficiencies of these vitamins may be classified into 3 broad categories: decreased intake, impaired absorption and increased requirements. In general, folate deficiency is most often the result of decreased intake and is more common in developing and socioeconomically distressed countries. Situations in which inadequate intake is further compounded occur when there is an increased folate requirement. Such situations arise in physiologic conditions including pregnancy, lactation, and prematurity as well as among populations in which there is a high prevalence of chronic hemolytic anemias, typically caused by hemoglobinpathies, most notably sickle cell anemia and the thalassemias. Other conditions associated with increased cell turnover such as leukemias, aggressive lymphomas, and other tumors associated with a high proliferative rate can also cause increased folate demand (3). Generalized exfoliative dermatitis also causes folate deficiency through increased loss of folate, as does hemodialysis. Causes of decreased intake include poor nutrition, old age, poverty, and alcoholism. Less commonly, inadequate intake can arise through inappropriate and prolonged hyperalimentation or other use of synthetic diets as well as the use of goat milk, which is low in folate. Although less common, impaired absorption may be a cause of folate deficiency, and it occurs in a variety of small intestinal diseases that include both tropical and nontropical sprue (adult celiac disease) and inflammatory bowel disease. Congenital selective malabsorption of folate is a rare but important disorder that has been found to be caused by mutations in the recently described proton-coupled folate transporter (3). As a matter of public health, the widespread implementation of folic acid fortification programs in many countries around the world has drastically reduced the prevalence of folate deficiency in those countries (4) and has tilted the distribution of causes of deficiency away from inadequate intake and toward increased requirements.

With respect to vitamin B-12, it is important to distinguish between low vitamin B-12 status ("subclinical deficiency") and outright vitamin B-12 deficiency. Low vitamin B-12 status denotes a condition in which laboratory tests indicate depletion of vitamin B-12 stores as judged by being outside of the normal reference range. In the case of direct measures of vitamin B-12 [plasma or serum vitamin B-12 or holotranscobalamin (holoTC)], low vitamin B-12 status is indicated by being below the lower limit of the reference range (for vitamin B-12, $<$200 pg/mL or $<$148 pmol/L; for holoTC, $<$35 pmol/L), whereas for indirect measures of metabolites (methylmalonic acid or homocysteine), low vitamin B-12 status would be indicated by a level above the upper limit of the reference range (for methylmalonic acid, $>$260 nmol/L; for homocysteine, $>$12 μmol/L). The distinction between low vitamin B-12 status and outright vitamin B-12 deficiency is not directly attributable to the actual measured concentrations of circulating vitamin B-12 or associated metabolites but is made on the basis of on the presence or absence of morbidity that is attributable to the vitamin B-12 deficiency state. Strictly, this also requires evidence of alleviation or arrest of progression of the clinical signs and symptoms of presumed deficiency on repletion of vitamin B-12. Individuals who develop outright vitamin B-12 deficiency with associated morbidity do, of necessity, transition through a stage of low vitamin B-12 status during the evolution of their disease. However, there are large numbers of individuals with low vitamin B-12 status who do not progress to frank deficiency. A logical explanation for this dichotomy relates to the degree of impairment of the process of assimilation and absorption of vitamin B-12 in relation to the daily requirement for the vitamin. Complete abrogation of physiologic vitamin B-12 absorption, such as occurs after total gastrectomy, ileal resection, or advanced autoimmune pernicious anemia, will inexorably lead to a degree of depletion of the vitamin that can no longer sustain cellular requirements and that would, with time, lead to both functional and structural abnormalities. However, in other situations, best exemplified by the so-called food vitamin B-12 malabsorption states, the basic mechanism of intrinsic factor-dependent vitamin B-12 absorption remains intact, but some aspect of the assimilative process is impaired, as in nonimmune atrophic gastritis or with the chronic use of proton pump inhibitors. The apparent ability to reabsorb the substantial amount of vitamin B-12 excreted in the bile in such individuals, as well as in vegetarians, further enables such individuals to maintain a precarious balance of adequacy of vitamin B-12 to satisfy critical cellular needs and thus avoid the major consequences of deficiency (2). Still, such individuals with depleted vitamin B-12 stores are clearly more vulnerable to becoming frankly vitamin B-12 deficient. There is uncertainty and ongoing debate as to whether low vitamin B-12 status per se may be associated with subtle degrees of deficiency that have consequences of public health significance.

With respect to causation of vitamin B-12 deficiency, a distinction can be drawn between causes of low vitamin B-12 status and outright vitamin B-12 deficiency as outlined above. Unlike folate, vitamin B-12 deficiency (as distinct from low vitamin B-12 status) is usually the result of malabsorption and rarely results only from dietary insufficiency, except as occurs in individuals who are strict vegetarians or vegans who shun all animal products. Because of the complex mechanism of vitamin B-12 absorption, causes of malabsorption may arise at several levels in the gastrointestinal tract (2, 3). At the gastric level, the most frequent cause of significant vitamin B-12 malabsorption leading to deficiency is the autoimmune disease pernicious anemia, which most often affects older individuals. Estimates of the prevalence of pernicious anemia among individuals aged

≥65 y in Western countries vary between 1% and 2% (3, 5). Less common gastric causes of vitamin B-12 deficiency include gastrectomy and congenital deficiency of intrinsic factor (2). Intestinal causes of vitamin B-12 deficiency include ileal resection or disease, stagnant intestinal blind loops and infestation with the fish tapeworm, Crohn disease, and tropical sprue. In young children, the uncommon inherited disorders of selective vitamin B-12 malabsorption caused by defects in the receptor for the vitamin B-12–intrinsic factor complex (Imerslund-Grasbeck syndrome) as well as congenital transcobalamin deficiency can give rise to vitamin B-12 deficiency (2). There are various conditions that impair but do not totally abrogate vitamin B-12 absorption. These give rise to varying degrees of low vitamin B-12 status and include simple atrophic gastritis (food vitamin B-12 malabsorption), hypergastrinemia caused by gastrin-producing tumors (Zollinger-Ellison syndrome), gastric bypass surgery for morbid obesity, and the use of proton pump inhibitors. Intestinal causes include gluten-induced enteropathy (nontropical sprue), chronic pancreatic insufficiency, HIV infection, pelvic radiation therapy, and graft-versus-host disease. A number of drugs can interfere with vitamin B-12 absorption and are reviewed extensively elsewhere (3). Of these, the only ones that are ingested over a period long enough to potentially deplete vitamin B-12 stores are the oral antidiabetic agents metformin and phenformin.

TESTS FOR ASSESSING FOLATE AND VITAMIN B-12 STATUS

There are several biomarkers that are currently used to assess folate and vitamin B-12 status. Various considerations that pertain to the usefulness, effect of confounders, cutoffs indicating deviation from normality, and associated clinical correlates are summarized in **Table 1** for vitamin B-12 and in **Table 2** for folate.

The measurement of vitamin concentrations in the blood as a means to reliably identify and distinguish folate and vitamin B-12 deficiencies is influenced by several factors (3). With respect to measurement of vitamin B-12 in plasma, the vitamin is carried on 2 distinct binding proteins. One, transcobalamin, binds only a small portion of the total plasma vitamin B-12 (20–30%) but is responsible for delivery of vitamin B-12 to cells and is considered to be the functionally important fraction. The major portion of plasma vitamin B-12 is bound to the other binding protein, haptocorrin, which is essentially inert as far as vitamin B-12 delivery to cells is concerned, although it may reflect the general underlying state of vitamin B-12 stores. In recent years, assays have become available to measure the transcobalamin-associated fraction of vitamin B-12. Accumulating evidence indicates that this assay may enhance the predictive power for identifying vitamin B-12 deficiency, either alone or when used in conjunction with other available tests (6–9).

The major features and limitations of the total serum or plasma vitamin B-12 assay are summarized in Table 1. Sensitivity of plasma vitamin B-12 measurement for detection of vitamin B-12 depletion or deficiency is good overall, but specificity is poor, although the predictive value is improved by measurement of methylmalonic acid. The concentration of plasma vitamin B-12 falls relatively late in depletion leading to deficiency, and this limits the utility of isolated vitamin B-12 measurement. There are several confounders that affect the concentration of circulating total vitamin B-12, which further limits the usefulness of the test without additional laboratory assessment. In situations of limited resource availability, however, vitamin B-12 measurement may be the only option for assessing vitamin B-12 status. For this purpose, microbiological assay of the vitamin can reliably be established in most laboratories. With respect to measurement of holoTC, changes in concentrations appear to respond sooner during depletion leading to deficiency. However, the overall predictive value of the test used in isolation does not appear to offer major advantage over the total plasma vitamin B-12 measurement. Good congruency of holoTC with plasma methylmalonate has, however, been reported, and the holoTC assay used in conjunction with the total plasma vitamin B-12 may provide enhanced predictive power to identify true vitamin B-12 deficiency (9). The availability of the holoTC assay is currently somewhat limited.

With respect to folate, plasma (or serum) folate undergoes diurnal changes related to recent food intake. Red blood cell folate, however, shows constancy from day to day and accurately reflects longer-term body folate status in that it reflects the average folate content of the long-lived circulating red cell population. In individuals, single measurement of plasma or serum folate alone does not allow for the distinction between a transient drop in folate intake and established chronic folate deficiency. On the other hand, folate in the plasma compartment is a reliable indicator of dietary folate intake. In interventional studies, single measurements of plasma or serum folate are not as reliable for assessing folate status as are red cell folate measurements. However, repeated plasma folate measurements over time in the same individual reflect trends in change of folate status. Furthermore, for purposes of population surveys, plasma folate measurement provides a suitable assessment of general folate status.

More recently, the possible importance of serum unmetabolized folic acid has come under scrutiny. The reason for this is that varying quantities of serum unmetabolized folic acid have been detected in healthy individuals residing in countries who practice folic acid fortification of the diet or in individuals who consume folic acid supplements (10). This oxidized and "unnatural" form of folate may be associated with deleterious effects on folate and vitamin B-12–dependent metabolic pathways. Measurement of serum unmetabolized folic acid requires either differential microbiological assay using microorganisms with different growth response requirements or HPLC with electrochemical detection. Either method is available only in highly specialized laboratories.

The alternative approach for assessment of folate and vitamin B-12 status involves surrogate measurement of metabolites known to increase in folate and vitamin B-12 deficiencies and provides certain advantages over direct measurement of vitamin concentrations. Two metabolites, homocysteine and methylmalonic acid, are of greatest utility as indicators for detecting and discriminating between underlying metabolic insufficiency of folate and vitamin B-12 (11–13). Homocysteine requires both folate and vitamin B-12 for its disposal through 1 of its 2 major pathways of conversion. Consequently, plasma homocysteine concentrations rise in both folate and vitamin B-12 deficiencies. Methylmalonate, on the other hand, requires only vitamin B-12 for its conversion to succinate. Whereas folate and vitamin B-12 deficiencies both give rise to elevations of plasma homocysteine,

TABLE 1
Comparison of vitamin B-12 biomarkers[1]

Biomarkers	Serum/plasma vitamin B-12	Serum holoTC	Serum/plasma MMA
How useful is it for assessing exposure (intake)?	+	++	++
	Overall good sensitivity. Poor specificity. Predictive value improved when combined with holoTC or MMA. Not appreciably influenced by recent intake. Sensitivity and specificity are confounded by pregnancy, liver disease, high white cell count, renal disease.	Good sensitivity and specificity. Predictive value improved when combined with vitamin B-12. Measures "functional" component of serum vitamin B-12. Reflects recently absorbed vitamin B-12 and falls sooner in negative balance. May be used as a surrogate for vitamin B-12 absorption.	Very good sensitivity. Moderately good specificity confounded by renal impairment, bacterial overgrowth. Reverts to normal within 7–10 d of vitamin B-12 repletion.
How useful is it for assessing status (short-, long-term)?	+	++	++
	Better for assessing long-term than short-term status. Concentrations decrease relatively late in depletion.	Good for short-term status and appears to remain useful for long-term status as well	Good for both short-term and long-term status
How useful is it for assessing function?	+	+	+++
	Generally not useful except in extreme or well-established deficiency/depletion	Reliable to assess function in that it generally reflects and is congruent with MMA. Also useful as a surrogate of vitamin B-12 absorption function. May correlate with cognitive function and brain volume?	Directly reflects biochemical vitamin B-12 adequacy and status and is therefore a functional marker except when confounders are present
Determinants or confounders (eg, age, sex, pregnancy, lactation, infection, polymorphisms)?	Age (particularly in elderly), pregnancy and lactation, drugs, polymorphisms (haptocorrin, HC)	Age?	Age
	Renal disease	Pregnancy and lactation?	Pregnancy
	Liver disease	Polymorphism (TC 776C→T)	Renal disease
	High white blood cell count	Renal disease	Bacterial overgrowth
Accepted cutoffs indicating deficient/normal/excess states?	Subclinical deficiency: vitamin B-12 <300 pg/mL (<220 pmol/L)	TC <35 pmol/L[2]	>260 nmol/L deficient[2]
	Deficiency: vitamin B-12 <200 pg/mL (<148 pmol/L)	Cutoffs vary in different reports (eg, <22 pmol/L)	
Other relevant issues: modifiers of validity (eg, infection)	Assay standardization	Assay standardization	Assay standardization
	Sample processing and storage	Sample processing and storage	Sample processing and storage
Population vs individual?	Both	Both	Both
Applicable in resource-constrained situations (eg, technical, sample storage, cost)?	Limited	Very limited	Very limited
	Microbiological assay offers accurate results at low cost but requires manual handling and has limited throughput	Only one method in widespread use; requires ELISA capability or automated instrumentation	Expensive and requires special instrumentation (LC-tandem mass spectrometry (MS/MS) or GC-MS)
Multiple biomarkers needed?	Yes, sensitivity and specificity (predictive value) improved in conjunction with holoTC or MMA	No, but predictive value improved with vitamin B-12 or MMA	No, but improves the predictive value of vitamin B-12 or holoTC when used in conjunction
Other (clinical) information needed?	Yes	Yes	Yes
	Hematologic findings	Hematologic findings	Presence of renal impairment
	Neurological findings	Neurological findings	Evidence of intestinal bacterial overgrowth
	Dietary intake	Dietary intake	
	Presence of renal impairment	Presence of renal impairment	

[1] holoTC, holotranscobalamin; MMA, methylmalonic acid; HC, homocysteine; LC, liquid chromatography; MS/MS, tandem mass spectrometry; GC-MS, gas chromatography–mass spectrometry; TC, transcobalamin; ELISA, enzyme-linked immunosorbent assay; +, useful; ++, very useful; +++, extremely useful.
[2] Subclinical and clinical.

TABLE 2
Comparison of folate biomarkers[1]

Biomarkers	Serum folate	RBC folate	Serum unmetabolized folic acid	Plasma tHcy
How useful is it for assessing exposure (intake)?	++ Very sensitive to recent changes in intake but confounded by pregnancy, alcohol ingestion. Repetitive measurements over time may be necessary.	+ Not reflective of current intake but reflects longer term status and can be useful in conjunction with serum folate. Frequently low concentrations due to vitamin B-12 deficiency.	++ Quite reflective of current intake of fortified food/supplements but not of total folate	++ Good correlation with folate especially in nonelderly age groups
How useful is it for assessing status (short-, long-term)?	+ Not always low in folate deficiency, and low concentrations are not necessarily indicative of folate deficiency	++ Compromised sensitivity (normal concentrations in folate depletion) and specificity (low concentrations in vitamin B-12 deficiency)	Not useful	++ Responds to treatment in days but may take longer to return to normal. Note: paradoxical small increase in tHcy shortly after dosing with folic acid reported.
How useful is it for assessing function?	Probably not useful Specific folate form (bioactive form) at the tissue level will affect function	Probably not useful Specific folate form (bioactive form) at the tissue level will affect function	Under investigation Unclear whether serum unmetabolized folic acid plays a role in cancer development and/or promotion, immune response, etc	+++ A functional indicator in the absence of confounders
Determinants or confounders (eg, age, sex, pregnancy, lactation, infection, polymorphisms)?	Age? Pregnancy Alcohol consumption Smoking Drug use interactions (antifolates) Thalassemia SNPs (MTHFR 677 C→T)	Age? Vitamin B-12 Pregnancy Alcohol consumption Smoking Drug use interactions (antifolates) Thalassemia SNPs (MTHFR 677C→T)	SNPs in DHFR?	Age Sex Renal function Polymorphism: MTHFR Other B vitamins: vitamins B-12, B-6, B-2
Accepted cutoffs indicating deficient/normal/excess states?	Negative balance: serum folate <7 nmol/L (3 ng/mL) Subclinical deficiency: serum folate <10 nmol/L (increased Hcy) Reduced risk of NTD-affected pregnancy: serum folate >16 nmol/L (7 ng/mL)	Deficiency: RBC folate <305 nmol/L (140 ng/mL) Subclinical deficiency: RBC folate <340 nmol/L (increased Hcy) Reduced risk of NTD-affected pregnancy: 900 nmol/L (≈400 ng/mL)	None	>12 μmol/L B vitamin deficiency/ imbalance ? lower for females, children
Other relevant issues: modifiers of validity (eg, infection)	Assay standardization Availability of standard reference materials with certified values for "total folate"	Assay standardization Availability of standard reference materials with certified values for "total folate"	Availability of standard reference materials with certified values for SFA (currently only reference value available)	Assay standardization
Population vs individual?	Both	Both	Probably both	Both

(Continued)

TABLE 2 (*Continued*)

Biomarkers	Serum folate	RBC folate	Serum unmetabolized folic acid	Plasma tHcy
Applicable in resource-constrained situations (eg, technical, sample storage, cost)?	Very limited Serum needs to be frozen within days of collection For long-term storage, it needs to be frozen $< -40°C$ Microbiological assay offers accurate results at low cost, but manual handling required and has limited throughput	Very limited Whole blood needs to be processed and frozen within days of collection Need to generate accurate hemolysate Need to determine hematocrit (and serum folate) Microbiological assay offers accurate results at low cost, but manual handling required and has limited throughput	No Requires chromatographic separation from other folate forms	Plasma preference Continued production of tHcy in erythrocytes and export into serum during clotting; keep EDTA whole blood chilled until processed (maximum: 4–6 h)
Multiple biomarkers needed?	Yes (for unequivocal diagnosis of folate deficiency)	No (but serum folate is a plus)	No	Yes (for unequivocal diagnosis of folate deficiency)
Other (clinical) information needed?	Yes RBC folate Serum vitamin B-12 Hematologic findings Dietary intake	Yes Serum vitamin B-12 Possibly serum folate Hematologic findings Dietary intake	Yes Dietary intake	Yes Renal function (creatinine)

[1] RBC, red blood cell; tHcy, total homocysteine; SFA, serum folic acid; Hcy, homocysteine; NTD, neural tube defect; MTHFR, methylenetetrahydrofolate reductase; SNPs, single nucleotide polymorphisms; DHFR, dihydrofolate reductase; +, useful; ++, very useful; +++, extremely useful.

vitamin B-12 deficiency alone causes elevation of plasma methylmalonic acid (11–14). Other conditions can cause elevations of either homocysteine alone (hypothyroidism, vitamin B-6 deficiency), methylmalonic acid alone (intestinal microbial overgrowth), or both (renal failure). Absent other confounding causes, elevation of plasma homocysteine or methylmalonic acid provides a sensitive indication of folate or vitamin B-12 deficiencies (11, 12). Several other considerations concerning the utility of plasma homocysteine and/or methylmalonic acid assays are shown in Tables 1and 2 in relation to the biomarkers that are potentially useful for assessment of either vitamin B-12 or folate status. As with homocysteine, methylmalonic acid concentrations vary continuously with the degree of vitamin B-12 deficiency. Overall, there is an inverse correlation between plasma vitamin B-12 and methylmalonic acid. There is an inflection point in the curve, however, that describes the relation of vitamin B-12 to methylmalonic acid. This point may be used to determine the concentration of vitamin B-12 below which there is a clear detrimental metabolic effect of the deficiency (15).

In summary, reliable and accurate assessment of folate and of vitamin B-12 status is required to determine the prevalence of deficiencies of these vitamins in populations and is necessary for developing suitable strategies to prevent or remedy these nutritional problems. Deficiency of these closely related vitamins is responsible for a considerable global burden of chronic ill-health and impaired functionality. Deficiencies of the 2 vitamins are prevalent among all age groups and are known to cause or contribute to a variety of disease states. Detecting or confirming the presence of folate or vitamin B-12 deficiency, and distinguishing one from the other, depends critically on laboratory testing. Screening tests to determine the presence of folate or vitamin B-12 deficiency are used singly or in combination to establish the nutritional status and prevalence of deficiencies of the vitamins in populations. The choice of test or test combination to assess folate and vitamin B-12 status and to monitor the efficacy of intervention depends on several considerations, including reliability, the setting in which the tests are used, and the availability and cost of the tests (3, 14). Efficacy of intervention programs through the use of fortification or supplements is also best monitored using such laboratory tests. Laboratory indicators of vitamin B-12 or folate status typically involve measurement of either the total or a physiologically relevant fraction of the vitamin in the blood. Alternatively, or additionally, measurements of surrogate biochemical markers that reflect the metabolic function of the vitamins may be used. The most widely used of these metabolic markers are homocysteine for detection of either vitamin B-12 or folate deficiency and methylmalonic acid for detection of vitamin B-12 deficiency only. Frequently, individual tests lack sensitivity, specificity, or predictive value, and for this reason, testing strategies often include several tests used in combination.

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REFERENCES

1. Bailey LB. Folic acid. In: Zempleni J, Rucker RB, eds. Handbook of vitamins. Boca Raton, FL: Taylor and Francis, 2007:385–412.
2. Green R, Miller JW. Vitamin B12. In: Zempleni J, Rucker RB, eds. Handbook of vitamins. Boca Raton, FL: Taylor and Francis, 2007: 413–57.
3. Green R. Folate, cobalamin, and megaloblastic anemias. In: Kaushansky K, Beutler E, Seligsohn U, Lichtman MA, Kipps TJ, Prchal JT, eds. Williams hematology. 8th ed, Chapter 41. New York, NY. McGraw-Hill, 2010:533–63.
4. Berry RJ, Mulinare J, Hamner HC. Folic acid fortification. Neural tube defect risk reduction—a global perspective. In: Bailey LB, ed. Folate in health and disease. 2nd ed. Boca Raton, FL: CRC Press, 2010: 179–204.
5. Carmel R. Prevalence of undiagnosed pernicious anemia in the elderly. Arch Intern Med 1996;156:1097–100.
6. Hvas AM, Nexo E. Holotranscobalamin—a first choice assay for diagnosing early vitamin B-12 deficiency? J Intern Med 2005;257:289–98.
7. Lloyd-Wright Z, Hvas AM, Moller J, Sanders TA, Nexo E. Holotranscobalamin as an indicator of dietary vitamin B12 deficiency. Clin Chem 2003;49:2076–8.
8. Herrmann W, Obeid R, Schorr H, Geisel J. Functional vitamin B12 deficiency and determination of holotranscobalamin in populations at risk. Clin Chem Lab Med 2003;41:1478–88.
9. Miller JW, Garrod MG, Rockwood AL, et al. Measurement of total vitamin B12 and holotranscobalamin, singly and in combination, in screening for metabolic vitamin B12 deficiency. Clin Chem 2006;52: 278–85.
10. Morris MS, Jacques PF, Rosenberg IH, Selhub J. Circulating unmetabolized folic acid and 5-methyltetrahydrofolate in relation to anemia, macrocytosis, and cognitive test performance in American seniors. Am J Clin Nutr 2010;91:1733–44.
11. Allen RH, Stabler SP, Savage DG, Lindenbaum J. Metabolic abnormalities in cobalamin (vitamin B12) and folate deficiency. FASEB J 1993;7:1344–53.
12. Green R. Metabolite assays in cobalamin and folate deficiency. Baillieres Clin Haematol 1995;8:533–66.
13. Carmel R, Green R, Rosenblatt DS, Watkins D. Update on cobalamin, folate, and homocysteine. Hematology (Am Soc Hematol Educ Program) 2003;1:62–81.
14. Green R. Indicators for Assessing folate and Vitamin B12 Status and for Monitoring the Efficacy of Intervention Strategies. Food Nutr Bull 2008;29(suppl):S52–63.
15. Selhub J, Jacques PF, Dallal G, Choumenkovitch S, Rogers G. The use of blood concentrations of vitamins and their respective functional indicators to define folate and vitamin B12 status. Food Nutr Bull 2008;29(suppl):S67–73.