World Health Organization

Serum and red blood cell folate concentrations for assessing folate status in populations

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VMNIS | Vitamin and Mineral Nutrition Information System

Inside

Background

Folate is the general term for a water-soluble B vitamin naturally found in foods such as leafy vegetables, legumes, egg yolks, liver and some citrus fruits *(1)*. Folic acid is the synthetic form of this vitamin that is commonly added to fortified foods and found in supplements. The bioavailability of naturally occurring folate is less than that of folic acid *(2)*. This vitamin is essential for normal cell growth and replication. Folate and vitamin B_{12} deficiencies have been acknowledged as the most common causes of macrocytic anaemia *(3)*. In addition, poor folate status is associated with other negative health outcomes. For example, inadequate maternal folate status has been linked to abruptio placentae, pre-eclampsia, spontaneous abortion, stillbirth, preterm delivery, low birth weight *(4)* and serious congenital anomalies of the brain and spine, such as neural tube defects (NTDs) *(5)*.

Increasing awareness of the public health significance of insufficient folate intake has emphasized the need for identifying accurate biomarkers for large-scale assessment of folate status. Although folate is mainly stored in the liver, folate status can be assessed in serum, plasma, red blood cells or urine. Serum folate is considered an indicator of recent folate intake *(6)*, and a single measurement cannot be used to differentiate between a transitory decrease in dietary folate intake and chronic deficiency states. However, repeated low values of serum folate within an individual over the course of a month are indicative of low folate status or folate depletion *(7)*. Conversely, red blood cell folate concentrations respond slowly to changes in folate intake, because the erythrocytes, which have a 120-day lifespan, only accumulate folate during erythropoiesis *(6)*. Thus, red blood cell folate concentrations are useful to indicate long-term folate status.

Multiple factors influence folate status, including genetics such as the methylenetetrahydrofolate reductase (*MTHFR*) *677C→T* gene polymorphism, which is the gene encoding the MTHFR enzyme *(8)*; physiological status (e.g. age, pregnancy, lactation) *(9, 10)*; biological factors (e.g. coexisting vitamin B_6 and B_{12} status, homocysteine levels) *(11)*; and contextual factors such as comorbidities (e.g. malaria) *(12)*, limited access to dietary folate sources and low socioeconomic status *(13)*.

Laboratory methods

Laboratory methods for measuring folate status were first developed in the 1950s *(14)* and these methods still form the basis for currently used assessment methods. Folate status can be assessed using a variety of techniques, including microbiological methods, protein-binding assays (e.g. radioisotope competitive binding and fully automated non-radioassays for clinical analysers) and chromatography-based assays (e.g. high-performance liquid chromatography coupled to various detectors, such as mass spectrometry) *(15)*.

The microbiological assay using *Lactobacillus rhamnosus* (previously known as *Lactobacillus casei*) has been recommended since 1968 for the measurement of folate, as it is responsive to multiple forms of folate species while excluding those without vitamin activity *(16, 17)*. However, the microbiological assay lacks specificity to differentiate between different forms of folate. Constant monitoring of the assay quality and regular use of reference preparations standards are necessary to check and maintain the accuracy in the results, particularly at lower concentrations *(16)*. A recent study compared microbiological assays from three laboratories and showed that different results were obtained, depending on the folate calibrator or microorganism used *(18)*. Protein-binding assays were found to be less accurate, with one measuring ~30% lower concentrations of red blood cell folate and serum folate relative to the microbiological assay and liquid chromatography-tandem mass spectrometry (LC-MS/MS) *(19, 20)*. This is considered to be due to under-recovery of 5-methyltetrahydrofolate, the predominant folate species in serum and red blood cells. Chromatography-based assays are able to differentiate between individual folate species. A 2010 expert round table on the use of folate assays in the United States (US) National Health and Nutrition Examination Survey (NHANES) endorsed the use of LC-MS/MS for serum folate but noted that use of the assay for red blood cell folate was premature at that time *(17)*.

The choice of laboratory tests depends, first and foremost, on the type of study being carried out, as well as the infrastructure and resources available. As such, the lack of highly experienced laboratory personnel and reliable technical support for instrumentation, as is often the case in low- and middle-income countries, may limit applications to simpler laboratory tests, such as the microbiological assay. However, other factors, such as the specimen volume and access to specific reagents and supplies, may also be limiting factors *(15, 21)*.

Scope and purpose

This document provides users of the Vitamin and Mineral Nutrition Information System (VMNIS) with guidance about the use of serum (or plasma) folate and red blood cell folate as indicators to assess folate status in different populations. It summarizes the current World Health Organization (WHO)-recommended thresholds used for defining folate status in populations, as well as the chronology of their establishment.

Assessment of serum folate and red blood cell folate concentrations is useful for monitoring trends in folate status and evaluation of the impact of public health interventions.

Description of technical consultations

This document synthesizes the current WHO guidelines, published previously in the following five documents:

- *Nutritional anaemias (22)*: report of a technical consultation of a WHO scientific group, held in Geneva, Switzerland, on 13–17 March 1967. This consultation followed two other consultations held in 1958 *(23)* and 1962 and focused on the etiology of nutritional anaemias and the feasibility of developing laboratory methods for field application. It was convened 3 years after the start of a multi-country collaborative study in India, Israel, Mexico, Poland, South Africa, the United Kingdom of Great Britain and Northern Ireland, the United States of America and Venezuela, which (i) investigated iron metabolism in pregnancy and the role of hookworm in anaemia during pregnancy; and (ii) further tested the procedures for examining blood and serum. The 1967 consultation reviewed the overall progress of the study and also discussed the nutritional requirements for iron, folate and vitamin B_{12}.
- *Nutritional anaemias (24)*: report of a meeting of a group of WHO experts, held in Geneva, Switzerland, on 11–15 October 1971. The group examined the validity of parameters and concepts in the field of nutritional anaemia and reviewed the information that had become available since the 1967 meeting. The topics covered in this meeting included: (i) standardization of techniques to measure folate status; (ii) studies on the availability and absorption of iron, folate and vitamin

B_{12}; and (iii) prevalence studies and trials on preventive measures in population groups.

- *Control of nutritional anaemia with special reference to iron deficiency (25)*: report of a joint meeting of the International Atomic Energy Agency (IAEA), the US Agency for International Development (USAID) and WHO, held in Geneva, Switzerland, on 28 October to 1 November 1974. The purpose of the meeting was to: (i) update the state of knowledge with regard to the etiology of nutritional anaemia; and (ii) provide guidance on some public health interventions considered useful for its control.

- *Conclusions of a WHO technical consultation on folate and vitamin B_{12} deficiencies (26)*: report of a meeting of a group of WHO experts, held in Geneva, Switzerland, on 18–21 October 2005. The consultation aimed to: (i) review the global prevalence, causes and health consequences of folate and vitamin B_{12} deficiency, with emphasis on the contribution of these deficiencies to the global burden of anaemia, adverse outcomes in pregnancy, child development and mental function, and cardiovascular disease; (ii) review the metabolism of folate and vitamin B_{12}; (iii) identify the best indicators for assessing folate and vitamin B_{12} status at the population level and monitoring the response to interventions; (iv) reach a consensus on cut-off values to define folate and vitamin B_{12} deficiency; (v) define criteria for determining the severity of folate and vitamin B_{12} deficiency at the population level that should trigger an intervention; and (vi) critically review current interventions (supplementation and fortification) to prevent folate and vitamin B_{12} deficiencies and their potential side-effects.

- *WHO Guideline: Optimal serum and red blood cell folate concentrations in women of reproductive age for prevention of neural tube defects (27)*: the WHO Department of Nutrition for Health and Development, in collaboration with relevant internal partners and guided by the WHO guideline development process, convened a guideline development meeting on 23–25 September 2013 in Geneva, Switzerland *(28)*, to discuss the evidence and reach consensus on recommendations for optimal blood folate concentrations in women of reproductive age for the prevention of NTDs. Cut-off values for folate had previously focused on the prevention of megaloblastic anaemia in all age groups. However, it has been recently recognized that blood folate concentrations in women of reproductive age may need to be higher to help prevent NTD-affected pregnancies. Thus, the group aimed to: (i) interpret the evidence for establishing cut-off values for folate in serum and red blood cells for women of reproductive age at the individual and public health level, with explicit consideration of the overall balance of risks and benefits; (ii) formulate final draft recommendations; (iii) determine the strength of these recommendations, considering the balance of evidence for benefits and harms, and taking into account costs, values and preferences; and (iv) define implications for research in the areas discussed. The guideline *(27)* examined four questions: (i) in the absence of an intervention, what are the key genetic, biological and sociodemographic determinants of folate status (i.e. serum, plasma or red blood cell folate) in women of reproductive age?; (ii) what is the threshold concentration of blood folate associated with the lowest probability/risk (depending on the statistical method) of having a NTD-affected pregnancy?; (iii) do blood folate concentrations respond to interventions to improve folate status in women?; and (iv) does the performance of the laboratory assays used to measure folate concentrations affect serum and red blood cell folate readings?

Recommendations

Cut-off values for the assessment of folate status in all age groups, using serum or red blood cell folate concentrations, were first proposed in 1968 *(22)* (see Table 1). Values indicative of deficiency were based on the concentrations at which macrocytic anaemia is more likely to appear. These cut-off values were endorsed by subsequent WHO consultations in 1972 *(24)* and 1975 *(25)*, although it was acknowledged that the correlation between folate concentration and megaloblastic anaemia was not always strong. The consultation highlighted the urgent need for data on the clinical significance of low concentrations of folate and vitamin B_{12} in non-pregnant individuals who have no evidence of other haematological changes, because studies at that time failed to detect any obvious impairment of health. It should be noted that folate concentrations defined as "elevated" were based on the assay's upper-limit capabilities without dilutions, and not on the biological implications for health.

In 2005, the cut-off values were revised to reflect folate deficiency based on metabolic indicators *(26, 29)* (see Table 2). These cut-off values were based on NHANES III data on American men and women aged

30 years and older. The study assessed the relationship between homocysteine and plasma or red blood cell folate, and identified the cut-off value for folate deficiency as the folate concentration below which homocysteine concentrations start to rise *(30)*. High levels of circulating homocysteine are considered a functional indicator of folate deficiency and are thought to result from the inability of folate to donate the methyl group necessary to convert homocysteine to methionine *(31)*. These values apply to all age groups, although the consultation recognized that the values may not be appropriate to assess folate status in pregnant women, since folate concentrations typically decline during pregnancy, nor are they adequate thresholds for the prevention of NTDs *(27)*. In 2015, guidelines were established for the determination of optimal folate status among women of reproductive age to prevent NTDs *(27)* (see Table 3). In this group, at the population level, red blood cell folate concentrations should be above 400 ng/mL (906 nmol/L) to achieve the greatest reduction of NTDs. This same threshold can also be used as an indicator of folate insufficiency in women of reproductive age at the population level. It should not be used as a threshold to predict the individual risk of having a pregnancy affected by a NTD, as low folate is not responsible for all NTDs. No serum folate threshold is recommended for prevention of NTDs in women of reproductive age at the population level.

Since 1968, the microbiological assay using *Lactobacillus rhamnosus* has been recommended for folate measurement and this assay continues to be recommended as the most reliable choice to obtain comparable results for red blood cell folate across countries *(27)*. However, use of different folate calibrators or different microorganisms may lead to different results among microbiological assays and laboratories, and may necessitate an adjustment of the threshold value for optimal red blood cell folate.

Table 1
Folate concentrations in serum and red blood cells for determining folate status in all age groups, using macrocytic anaemia as a haematological indicator

Serum/plasma folate levels ng/mL (nmol/L)[a,b]	Red blood cell folate level ng/mL (nmol/L)[a,b]	Interpretation
>20 (>45.3)		Elevated
6–20 (13.5–45.3)		Normal range
3–5.9 (6.8–13.4)		Possible deficiency
<3 (<6.8)	<100 (<226.5)	Deficiency

[a] Folic acid conversion factor: 1 ng/mL = 2.265 nmol/L.
[b] Assayed by *Lactobacillus casei*.
Source: reference *(22)*.

Table 2
Cut-off values for determining folate deficiency in all age groups, using homocysteine concentrations as metabolic indicator

Folate indicator	Cut-off value indicating folate deficiency ng/mL (nmol/L)[a,b]
Serum/plasma folate level	4 (<10)
Red blood cell folate level	<151 (<340)

[a] Folic acid conversion factor: 1 ng/mL = 2.265 nmol/L.
[b] Measured by the radioimmunoassay. In this dataset, the measurements obtained by radioimmunoassay need adjustment to make them comparable with the microbiological assay *(29)*.
Source: reference *(26)*.

Table 3
Folate concentrations in red blood cells for preventing neural tube defect-affected pregnancies in women of reproductive age at the population level[a]

Red blood cell folate level, ng/mL (nmol/L)[b,c]	Interpretation
> 400 (>906)	Folate sufficiency
< 400 (<906)	Folate insufficiency

[a] These thresholds should not be used at an individual level for determination of risk of a neural tube defect-affected pregnancy.
[b] Folic acid conversion factor: 1 ng/mL = 2.265 nmol/L.
[c] No serum folate threshold is recommended for the prevention of neural tube defects in women of reproductive age at the population level.
Source: reference *(27)*.

Summary of statement development

The main bibliographic sources used to prepare this summary were five WHO publications released between 1968 and 2015 *(22, 24–27)*. All of these reports have contributed to building knowledge in this area. Briefly, cut-off values for folate deficiency based on risk of megaloblastic anaemia were first presented in the 1968 document *(22)*. The cut-off values were revised at a consultation held in 2005, using homocysteine as a metabolic indicator of deficiency *(26)*. In 2015, red blood cell folate thresholds for the prevention of NTDs among women of reproductive age at the population level were established *(27)*.

Plans for update

The WHO Evidence and Programme Guidance Unit, Department of Nutrition for Health and Development, is responsible for reviewing this document and, if needed, for updating it by 2025, to include any evidence-informed guideline developed in this topic,

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