

N08028: Informed systematic review and critical comparison of analytical methods for the quantification of blood folate status in the population

Executive Summary

Background:

The Scientific Advisory Committee on Nutrition (SACN), Folate Subgroup recently evaluated the scientific evidence of the merits and disadvantages of introducing mandatory fortification of flour with folic acid in the UK. If fortification is introduced by the UK Government, careful and accurate monitoring of folate status in the UK population must be initiated in order to allow precise determination of the impact of fortification on folate levels in the general population and specifically in “at-risk” groups such as the very young and the elderly. To ensure careful population monitoring, SACN recommended that a standard analytical method for measuring folate status be applied to nutritional surveys. To do this, the suitability of each of the methods currently employed was assessed.

Aims:

- (1) Critically assess the suitability of established methods for quantifying folate status.
- (2) Recommend the best method for future monitoring of folate status in the UK population.

Approach:

- (1) Literature review: A broad review of the published literature assessing the suitability of established methods for quantifying folate status in people was carried out. Comments and insights from experts consulted throughout the review process outlining less well-documented problems associated with each assay, together with unpublished data on assay robustness and reproducibility, was included in this document which was circulated to all delegates in advance of an expert/stakeholder workshop.
- (2) Consultation exercise: delegates were sent a list of potential topics for discussion at the expert workshop based on issues raised by the literature review. Delegates were asked to confirm whether these issues were relevant and were invited to add topics for debate. Feedback was collated and all issues debated at the workshop.
- (3) The utility of each method was discussed at an expert and stakeholder workshop held in London in April 2008. Delegates were experts either in (1) folate methodology, (2) quantifying folate in human studies and population monitoring or (3) developing quality control and international standards for accurate quantification of folate in blood.

Recommendations:

1. In order to accurately establish blood folate status in the NDNS, both red cell and serum folate must be measured.

2. Fasted samples must be collected to facilitate true measurement of folate status in the NDNS. One possible exception is samples collected from young children.
3. Protein-binding assays currently in clinical use are limited with respect to accuracy, linearity, reproducibility and continuity and may not facilitate accurate data interpretation with time. These should be discontinued for use in the NDNS.
4. LC MS/MS can provide data on total folate status, unmetabolised folic acid concentrations and specific folate vitamers in blood. Although not currently employed in the UK, LC MS/MS is an internationally-recognised reference method for quantifying folate with established International Standards (IS) and QA systems. In order to “future proof” the NDNS, LC MS/MS should be adopted as the principal method of choice for measuring folate status in the general population.
5. The microbiological assay, which accurately measures total folate, is a reasonable second choice if feasibility and cost-benefit analysis shows the use of LC MS/MS to be impractical.
6. Whichever method is chosen, the NDNS contract for measuring folate status must go to an established laboratory with experience in the appropriate technology, troubleshooting and application of QA controls.
7. The chosen method must be enrolled in at least one Quality Assurance scheme (such as UK NEQAS). International Standards quantified and validated by LC MS/MS must be used routinely to ensure accuracy and reproducibility.
8. Optimum sampling and storage protocols must be established and adhered to where at all possible. Serum, rather than plasma should be collected. The field laboratory (where samples are collected) should provide only short-term storage (up to 1 week) at 4°C of both whole blood and serum samples before shipment to an experienced “receiving laboratory” for processing. Samples should not be posted at room temperature. If optimal storage and shipping protocols cannot be guaranteed, blood can be treated with ascorbic acid (AA) at the field laboratory to retard degradation. Samples must be stored in the longer-term (> 2 months) at -80°C.
9. The effect of increasing folic acid intake on free folic acid blood concentrations in serum must be quantified pre- and post-fortification. A mechanism should be put in place to gather data on the kinetics and metabolism of synthetic folic acid from fasted and non-fasted blood samples. This could be conducted separately from the NDNS, although the possibility of using serum samples from this and other surveys (such as the Annual Health Survey) should be investigated.
10. Sub-groups of the population potentially at risk from the effects of elevated circulating concentrations of unmetabolised folic acid (e.g. young children and the elderly), and groups where fortification could be most effective (e.g. women of child bearing age) should be over-sampled pre- and post-fortification. This should be primarily to establish the effects of mandatory fortification on folate status rather than on disease risk. The FSA must take

advice on how best to ensure that sampling sub-groups is adequately statistically powered.