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Reply to S Ferraro and M Panteghini

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Dear Editor:

The commentators Ferraro and Panteghini (1) have highlighted issues in the folate field surrounding the impact of folate assay types and the preferences that exist between groups, across time, and between public health and clinical environments. The objective of Chen et al. (2) was not to determine a cut-off for plasma folate concentration insufficiency that is universal to any clinical laboratory assay type; the objective was to determine an equivalent of the RBC folate threshold of 906 nmol/L in a well-controlled population-based trial and to define biological factors that may impact the association between RBC folate and plasma folate. The microbiologic assay (MBA) is required for meaningful interpretation of RBC folate concentrations because it does not have the significant biases observed with protein-binding assays typically used in clinical settings (3), and it is the only assay linked to the risk of neural tube defects (4). To examine the association between RBC folate and plasma folate concentrations it is important to utilize the same assay in order to minimize sources of variability that might impact the association. Using the same method allowed us to clearly show differences between the 2 biomarkers in the presence of varying vitamin B-12 status and, to a lesser extent, other factors. The 2 folate biomarkers represent different biological "pools": in one, folate circulates in the blood, whereas in the other, folate is processed into the cell and is available for use as represented by the RBC folate concentrations. The substantial differences in plasma folate cut-off with vitamin B-12 insufficiency and deficiency compared with adequate vitamin B-12 status limit the utility of plasma folate concentrations to interpret the risk of neural tube defects. This also suggests that to increase RBC folate concentrations it may be advisable to increase vitamin B-12 status and that discordant RBC folate and plasma folate concentrations may be of biological and clinical interest. If different assays were used for RBC folate and plasma folate, it would be difficult to attribute the differences to biological phenomena compared with different assay artifacts. Additionally, as stated in the WHO guideline, the 906 nmol/L threshold is not intended for individual-level risk prediction (i.e., clinical setting) but is for population-level monitoring; the same would apply to a serum/plasma threshold based on the RBC folate threshold. It is noteworthy that the clinical recommendation is for all women capable of becoming pregnant

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to consume 400 (μg/d of folic acid from supplements or fortified foods; the recommendation does not include prepregnancy folate testing.

Ferraro and Panteghini raise important points, namely the difficulty in transferring cut-off values across assays (5) and in comparing folate data across studies (6). Although the standardization of folate assays is the ultimate goal for both clinicians and public health officials, the complexities surrounding folate measurements (e.g., multiple folate forms with most but not all having biologic activity, suboptimal stability of reduced folates, the need to deconjugate folate polyglutamates in RBCs) resulted in slow progress toward standardization, with assay comparability still being poor. Of note, the contemporary MBA provides acceptable imprecision (10%) and the laboratory procedure is much simpler compared with the traditional assay (e.g., use of cryopreserved microorganism inoculum, no need to use aseptic conditions, miniaturization to 96-well plate format) (7). More importantly, the contemporary CDC MBA calibrated with 5methyltetrahydrofolate produces comparable serum folate results to the CDC LC-tandem MS assay (LC-MS/MS): CDC MBA = 1.0005 × CDC LC-MS/MS + 0.1776 nmol/L, $r^2 = 0.9975$, $n = 23$ (8). Furthermore, the international collaborative study that evaluated the WHO International Standard 03/178 also showed good agreement between the LC-MS/MS conducted in 2 laboratories (5.33 ng/mL) and the MBA conducted in 3 laboratories (5.55 ng/mL) (9). These reasons, in addition to the relatively low assay cost and simple instrumentation needs, have led to a renewed interest in the MBA, specifically for the purpose of population folate status assessment as part of micronutrient surveys (10). It is clear, however, that even the contemporary MBA is not a suitable tool for the clinical laboratory setting, which requires fully automated, high-throughput, and fast-turnaround assays.

Lastly, we wanted to mention an issue that sometimes leads to confusion in the literature. Reference intervals delineate “normal” compared with “unusual” concentrations. They are derived through a statistical approach by using tail percentiles in the concentration distribution of a clearly defined population. Reference intervals are useful in the public health setting to evaluate the population distribution and shifts in the population distribution. Reference intervals are also often used to interpret folate concentrations in the clinical setting. However, one needs to recognize that the concept of reference intervals is different from the concept of a clinical or medical decision point (cut-off) based on a specific health outcome (11). For example, through experimental folate depletion investigators have defined stages of deficiency with megaloblastic anemia being the final stage of deficiency based on a hematologic indicator (12). A serum folate concentration of <3 ng/mL (<7 nmol/L) indicated a negative balance measured by the traditional MBA. We cannot expect that the lower end of the reference interval coincides with this serum folate deficiency cut-off value. However, the 2 values should be reasonably close in a healthy, folate-replete population.

In summary, the objective of Chen et al. was met with the appropriate assay in agreement with WHO recommendations. The generalizability and utility of these cut-offs across assays and outcomes are limited and this is documented in the literature and recommendations.

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