Letter to the Editor

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Estimation of the reference interval for serum folate measured with assays traceable to the WHO International Standard

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To the Editor,

Investigating the harmonization of folate immunoassays, Kristensen et al. recently reported for most commercial assays acceptable measurement differences within the limit of desirable bias [1]. This is probably due to the assay recalibration to the WHO International Standard-National Institute for Biological Standards and Control (NIBSC) code 03/178 [2]. However, we have recently experienced with the recalibration of Roche Diagnostics Folate III assay, now traceable to NIBSC 03/178 material, a significant shift in the average folate measured values. Particularly, at serum folate concentrations around the lower reference limit (LRL) of the old Roche assay (which was calibrated by manufacturer) we observed a positive bias of ~50% vs. the new Roche recalibrated assay. The regional external quality assessment program confirmed these results where, by cumulating data generated by participants using the Roche system, a dichotomous distribution of folate results became evident. Taking into account this difference, the shift from 4.6 μg/L (Roche recommended LRL for old calibration) to 3.9 μg/L (Roche recommended LRL for recalibrated assay) appears to be inconsistent. Here we report data from apparently healthy individuals obtained with the WHO-recalibrated assay in order to accurately define the traceable reference interval for serum folate. Without an adequate reference interval, the recalibration changes can indeed impair the interpretation of the folate results and, paradoxically, worsen the patient's outcome [3].

We derived the reference interval of serum folate in agreement with the Clinical and Laboratory Standards Institute C28-A3c standard [4], by enrolling a cohort of 322 healthy blood donors (50% males), signing an informed consent and filling in a nutritional questionnaire. Subjects were excluded if: a) hemoglobin (Hb) and erythrocyte mean corpuscolar volume (MCV) values exceeded reference limits for adults (females: 120–160 g/L, males: 130–175 g/L for Hb and 80–99 fL for MCV, respectively), b) supplemented with folic acid, or c) the hemolytic index of collected serum sample (estimated on a Abbott Architect c16000 system) was ≥25. After blood centrifugation, serum samples were immediately stored at –80 °C for a maximum of 3 months until folate determination using WHO-recalibrated Folate III assay (code 07559992190) on a Cobas 6000 analyzer (Roche Diagnostics). Measurements were performed in four runs using a single reagent lot (no. 123242) by checking the system alignment before and just after running samples by control materials provided by Roche (lot no. 18746200 and 18746300). The assay shows a total imprecision (as coefficient of variation) ≤7.2% at an average concentration of 6.5 μg/L (data from our internal quality control), a limit of detection of 0.6 μg/L and an upper limit of calibration range of 20 μg/L. Continuous variables with normal distribution according to the Shapiro-Wilk test were expressed as mean (±standard deviation), alternatively as median (25th–75th percentile), whereas dichotomous variables were expressed as absolute number and percentage. T-test or Mann–Whitney test when appropriate were used to compare groups and a multiple regression model (MRM) to estimate the influence of subject features (i.e. age, gender, Italian origin, smoking habit, portions of consumed fruit/vegetables) on serum folate concentrations. All statistical analyses were performed by R software.

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Table 1: Features of studied subjects.

	Overall (n=322)	Males (n=161)	Females (n=161)	Males vs. Females difference (p-Value)
Age, years[a]	45.4 (33.1–54.6)	45.8 (30.3–54.5)	45.2 (35.9–54.7)	0.50
Hemoglobin[a], g/L	142 (133–150)	150 (143–155)	134 (127–140)	<0.001
Mean corpuscolar volume[b], fL	87.7±3.9	87.2±3.8	88.0±3.9	0.05
Fruit and vegetable portions[a]	2 (2–3)	2 (1–3)	2 (2–3)	<0.001
Smoking	76 (23.6%)	43	33	0.20
Non-Italian	8 (2.5%)	3	5	0.47
Folate[a], µg/L	4.1 (2.9–5.6)	3.9 (2.9–5.3)	4.3 (2.9–6.0)	0.22

[a]Median (25th–75th percentile). [b]Mean (± standard deviation).

Table 1 reports the main features of enrolled subjects. Our results showed a reference interval, defined by 2.5–97.5th percentile limits (90% confidence interval), of 1.3 (1.1–1.4)–9.8 (8.6–12.2) µg/L. In particular, the LRL was markedly lower than the one previously estimated by us using the old Roche assay on a similar population (i.e. 3.3 µg/L). Since folate resulted in a positive skewed distribution, the concentrations followed logarithmic transformation before entering in the MRM. According to the MRM results (adjusted R^2, 18.9%; $p<0.001$), the number of portions of fruit and/or vegetables consumed per day was evidenced as the main factor significantly influencing folate values. In particular, subjects consuming three to four portions had higher median values (4.8 and 5.2 µg/L, respectively) than those consuming one to two portions (3.4 and 4.0 µg/L, respectively) ($p<0.001$). Furthermore, we confirmed the evidence of folate increase with age ($p<0.001$), whereas we found no effect by gender ($p=0.40$), non-Italian origin ($p=0.80$) and smoking ($p=0.80$) on serum folate concentrations in contrast with previous data showing higher concentrations in females and non-smokers [5].

In conclusion, our experimental estimate of LRL using Roche WHO traceable assay on a population free of folate supplementation has revealed that this value is far lower than that reported by the manufacturer in the assay package insert, likely including subjects from fortified populations. Notably, laboratories using folate assays harmonized to NIBSC 03/178 material may adopt the LRL of 1.3 µg/L to detect vitamin deficiency, providing that there are no differences in test results across populations due to biological or environmental factors.

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