METHOD VALIDATION OF SERUM/RBC FOLATE USING MICROBIOLOGICAL ASSAY

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Background:
Folates are water-soluble vitamins responsible for cellular development, metabolism and hematopoiesis. According to the DOST-FNRI’s National Nutrition Survey (2008), the prevalence of folate deficiency among Filipino women of reproductive age was 38.7% and 20.9% based on serum and red blood cell folate, respectively, using radioimmunoassay kits. Since folate deficiency is associated with megaloblastic anemia and neural tube defects, it is necessary to continually assess the folate status of the population in order to initiate an intervention. However, production of the kit has been discontinued, thus, the validation of the microbiological assay (MBA) using Lactobacillus rhamnosus was conducted.

Objective:
The study aimed to validate the folate microbiological assay for serum/RBC folate.

Materials and Methods:
Microbiological assay of folate in serum and whole blood hemolysate was done by absorbance readings in a microplate reader at 590 nm. Cryoprotected L. rhamnosus (ATCC 2773) was prepared and dispensed in multiple vials for long-term storage. Several calibration and optimization runs were performed using 5-methyltetrahydrofolic acid (5MeTHF) and certified reference materials (CRM) for whole blood hemolysate and serum, respectively.

Results and Findings:
Using the 35% coefficient of variation (CV) for serum and 50% CV for whole blood hemolysate, acceptable range for the CRMs were computed at 12.14–16.3 nanomole per liter (nmol/L) and 29.5–44.3 nmol/L respectively. Initial trial showed very minimal growth of the microorganism. Calibration curve was established after anaerobic incubation. Results from the trials showed mean values within acceptable range (16.02 nmol/L for serum and 40.35 nmol/L for whole blood hemolysate) but repeatability and reproducibility merits were not met. Related studies suggest the use of standardized critical reagents and effective heat sealing of the microplates to prevent leakage and to improve precision.

Conclusion and Recommendation:
Initial trials of the MBA showed unsatisfactory results. Data obtained for the CRMs were within the computed acceptable range but with high CV. Improvement on the conduct of assay to achieve better calibration curve and folate measurements will be implemented using the frozen assay kits provided by the Centers for Disease Control and Prevention.