Value Assignment of Vitamin D Metabolites in Vitamin D Standardization Program (VDSP) Serum Samples

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The number of commercial and laboratory-developed measurement platforms for 25-hydroxyvitamin D [25(OH)D] continues to grow, yet inter-method variability has been cited as a significant obstacle to establishing optimal levels of vitamin D exposure. One of the primary objectives of the Vitamin D Standardization Program (VDSP), an international collaborative effort initiated by the National Institutes of Health’s Office of Dietary Supplements (NIH-ODS), is the standardization of total 25(OH)D measurements over time, location, and laboratory procedure. Standardization of these measurements is necessary for comparing data across different populations and research studies, and for ensuring appropriate decision making by medical professionals.

One of the first projects undertaken by the VDSP was the value assignment of total 25(OH)D, meaning the sum of 25(OH)D₂ and 25(OH)D₃ concentrations, in 50 single donor serum samples. These assigned values serve as the foundation for several aspects of the VDSP, including a survey of the performance of assays for total 25(OH)D, evaluating the commutability of reference and quality assurance materials, and in developing study designs and statistical approaches for standardizing data from completed national health surveys. Concentrations of 25(OH)D were determined by two independent isotope-dilution liquid chromatography/tandem mass spectrometry (ID LC-MS/MS) methods developed by the National Institute of Standards and Technology (NIST) and by Ghent University. Both methods are recognized as reference
measurement procedures by the Joint Committee for Traceability in Laboratory Medicine (JCTLM). To our knowledge, this is the first time that two reference measurement procedures have been used to assign values to such a large number of serum samples.

Values for 25(OH)D$_2$ and 25(OH)D$_3$ were assigned to each of the 50 samples based upon the average of the averages of determinations made by NIST and Ghent. Results for 3-epi-25(OH)D$_3$ were based solely on the average of results from NIST. For 25(OH)D$_2$, 17 of the 50 samples had concentrations that were above the limit of quantitation (LOQ) for both laboratories. Values for total 25(OH)D were based upon the sum of 25(OH)D$_2$ and 25(OH)D$_3$ and included those values for 25OHD$_2$ that were below the LOQ. Results from each laboratory for SRM 972 Vitamin D in Frozen Human Serum were used to evaluate bias, and none of the biases differed significantly from zero. An uncertainty evaluation was also performed for each of the assigned values, including 25(OH)D$_2$, 25(OH)D$_3$, and total 25(OH)D, and the median coefficient of variation for total 25(OH)D was 1.8%.