

The 2013 ABVD-A challenges were composed of serum samples obtained from several donors, some of whom had been supplemented with vitamin D2 (with IRB approval), with minimal sample processing. The Survey offers laboratories an opportunity to compare their measured results with those obtained by the Centers for Disease Control and Prevention Reference Laboratory, which uses an LC-MS/MS methodology for measurement of vitamin D. The use of donor samples with minimal processing is intended to greatly reduce or eliminate matrix effects that may be seen with some proficiency testing materials.

Results are provided in this PSR for total 25-OH vitamin D, 25-OH vitamin D2, and 25-OH vitamin D3 by methods reported by participating laboratories. The reference target values provided by the CDC are shown for each sample.

Naturally occurring vitamin D in humans is composed of vitamin D3 (cholecalciferol), which is synthesized in skin on exposure to UV light. Vitamin D2 (ergocalciferol) is obtained from plant sources. Over-the-counter supplements may contain either form. Prescription formulations used to raise vitamin D levels usually contain vitamin D2. It is important that clinical assays used to assess vitamin D stores are capable of measuring both D2 and D3 because both are biologically active. In most clinical settings, measurement of 25-hydroxy vitamin D provides an adequate assessment of vitamin D stores. Measurement of 1,25-dihydroxy vitamin D, which is the most active form, is needed only rarely. Immunoassays usually report total 25-hydroxy vitamin D, whereas methods based on LC-MS/MS should be able to separately quantify both D2 and D3. Depending on chromatographic conditions, LC-MS/MS may also detect the C3-epimer, which is of uncertain biological significance, but tends to be a more prominent form of vitamin D in newborns.

Grading for this Survey remains unchanged: for total 25-OH Vitamin D, acceptable performance requires a value within 25% of the CDC reference value; although no formal grading is done for 25-OH Vitamin D2 or for 25-OH Vitamin D3, participants can compare their results to the target values.

As reflected in the reference values, sample ABVD-01 contained a relatively high concentration of 25-OH D2 (23.5 ng/mL) in relation to total 25-OH D (41.5 ng/mL). Samples ABVD-02 and ABVD-03 consisted of almost entirely 25-OH D3 (25-OH D2 in each was <1.0 ng/mL). These differences were reflected in several different commercial immunoassay system peer group means. For sample ABVD-01, the mean total 25-OH vitamin D values reported by users of the Abbott Architect i, Roche Cobas e411/elecsys and Roche Cobas e600/e170 instruments were approximately 25% lower than the reference value. These same systems did not demonstrate a bias of this magnitude in samples ABVD-02 and ABVD-03. These assays appear to underestimate vitamin D2. Underestimation of vitamin D may lead to unnecessary prescription or administration of vitamin D, with potential risks to patients of vitamin D toxicity. Conversely, the same type of analysis of the data suggests that the Siemens Advia Centaur overestimates vitamin D2 by about 20%. The importance of these biases is expected to depend on the relative concentrations of D2 and D3 in a clinical specimen.

Laboratories should compare their results to the CDC target values well as to their own peer group (if available). The purpose of these comparisons is to show you whether observed differences are local to your laboratory or are also seen by other users of your method. For example, if you have a value close to the mean of your peer group but very different from the true value that probably reflects a problem with the peer group method rather than with how your laboratory is running the assay.

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1. Participant Summary Report from Survey 2009 Y-A of the College of American Pathologists. Available at http://www.cap.org/apps/docs/committees/chemistry/measurements_25_OH_vitamin_d.pdf
2. Ross AC, Manson JE, Abrams SA, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. *J Clin Endocrinol Metab.* 2011; 96:53–58.
3. Farrell C-JL, Martin S, McWhinney B et al.: State-of-the-Art Vitamin D Assays: A Comparison of Automated Immunoassays with Liquid Chromatography– Tandem Mass Spectrometry Methods. *Clin Chem* 2012; 58:531-42.
4. Holick MF: Vitamin D deficiency. *New England Journal of Medicine* 2007; 357:266-281