



## **Educational Discussion: Vitamin D**

### **2016-A Accuracy Based Vitamin D Survey (ABVD)**

The 2016 ABVD-A challenges are a continuation of the previously established accuracy based program for vitamin D. The specimens included in the Survey were composed of pooled off-the-clot, fresh frozen serum specimens obtained from several donors, some of whom received oral vitamin D2 prior to their blood draw (under an IRB-approved protocol). Target values were established by the LC-MS/MS reference measurement procedure performed at the Centers for Disease Control and Prevention (CDC) Reference Laboratory. This Reference Laboratory also participates in the Vitamin D Standardization Program coordinated by the National Institutes of Health (NIH). Recently, in collaboration with the NIH's Vitamin D Standardization Program, specimens in the CAP's ABVD Survey were demonstrated to be commutable and fit-for-purpose for proficiency testing of LC-MS/MS assays and all clinical assays that were tested for the quantification of total vitamin D concentrations in human serum specimens. The minimal processing of the specimens prior to distribution was vital in making specimens that are commutable across assays. Results are provided in this Summary Report for total 25-OH vitamin D, 25-OH vitamin D2, and 25-OH vitamin D3 by measurement procedures used by participating laboratories. The reference target values provided by the CDC Reference Laboratory are also 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 and fungal sources. Most over-the-counter supplements now contain vitamin D3, although a few still contain vitamin D2. The only FDA-approved prescription formulation used to raise vitamin D levels contains only vitamin D2. In most clinical settings, measurement of total 25-hydroxy vitamin D provides an adequate assessment of vitamin D stores, and it is important that clinical assays used to assess vitamin D stores are capable of accurately measuring both D2 and D3 because both are biologically active. Measurement of 1,25-dihydroxy vitamin D, which is the most active form, is needed only rarely clinically. Immunoassays usually report total 25-hydroxy vitamin D, whereas measurement procedures based on LC-MS/MS can separately quantify both 25-OH D2 and 25-OH D3. Depending on chromatographic conditions, LC-MS/MS may also separately identify and measure the C3-epimer of 25-OH vitamin D3, which is of uncertain biological significance, but tends to be a more prominent form of vitamin D found in newborns' blood.

Grading criteria for this Survey remains unchanged: for total 25-OH Vitamin D, acceptable performance requires a value within 25% of the CDC reference value:



Specimen	CDC Target for Total 25-OH Vitamin D (ng/mL)	“Acceptable” Total 25-OH Vitamin D Range (ng/mL)	Method-specific Passing Rate % (Lowest/Highest)	All Methods Passing Rate %
ABVD-07	59.18	44.3 - 74.0	40.0/95.9	80.4
ABVD-08	14.52	10.8 - 18.2	34.5/91.3	74.5
ABVD-09	18.99	14.2 - 23.8	86.2/98.7	92.1

Pass rates listed are for methods with a peer group  $n \geq 10$ .

Although no formal grading is done for 25-OH Vitamin D2 or for 25-OH Vitamin D3, participants should compare their results to the CDC Reference Laboratory established target values.

For the three specimens in this Survey, many laboratories using LC-MS/MS did not report values within the acceptable range (within 25% of the target value), which is likely due to issues with calibration, variable sensitivity of the measurement procedures at low concentrations of 25-OH-D2 (e.g., <5 ng/mL), or due to concomitant detection of the C3-epimer of 25-OH D3. It is recommended that laboratories performing LC-MS/MS assays that did not obtain acceptable results consider the use of reference materials from National Institute of Standards and Technology (i.e., NIST SRM 972a) to confirm accurate calibration of their measurement procedures or consider the use of a chromatographic method that can resolve the 3-epimer in the analysis. Note that the CDC Reference Laboratory’s assigned values for total 25-OH vitamin D concentrations include only the sum of 25-OH vitamin D2 and 25-OH vitamin D3 concentrations, and do not include the measured concentration of 3-epimer of 25-OH vitamin D3. Although the 3-epimer was in fairly low concentration in all the three samples (5.6 ng/mL in ABVD-07, 0.9 ng/mL in ABVD-08, and 2.1 ng/mL in ABVD-09 as measured by the CDC Reference Laboratory), laboratories using LC MS/MS procedures that do not separate it from 25-OH vitamin D3 would tend to have a slightly high bias on both their total 25-OH vitamin D and 25-OH vitamin D3 compared to the CDC Reference Laboratory’s established target values.

Immunoassays and protein binding assays frequently have sample-specific interferences that can lead to variable performance. These interferences, which can include, but are not limited to, other vitamin D metabolites and certain lipids, lead to scatter around the regression of measurements using these assays compared to values from LC-MS/MS reference measurement procedures. Generally, vitamin D metabolites are correlated with one another and as a result, the calibration of immunoassays and protein binding assays might yield accurate results **on average**. However, due to scatter around the regression line, these assays could produce results that are more than 25% different than the reference measurement procedures **for a specific clinical sample**. This could be the reason that the Abbott Architect I measurement procedure reported significantly higher concentrations for total 25-OH-D in one of the specimens that contained predominantly 25-OH-D3 (ABVD-07), although calibration could also be an issue. As observed in previous ABVD Surveys, the Roche cobas e411/Elecsys and Roche cobas e600 series/e170 method groups produced results for ABVD-08 that were frequently more than 25% below the reference measurement procedures, demonstrating that these assays seriously under-recover 25-OH-D2. Other immuno/protein-binding assays that appear to under-recover 25-OH-D2 relative to 25-OH-D3 (the results of ABVD-08 are



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qualitatively different from ABVD-07 and ABVD-09) include the Beckman Unicel DXI, Diasorin Liaison, and Siemens Advia Centaur XP.

Laboratories should compare their results to the CDC target values as 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 measurement procedure generally rather than with how your laboratory is running it.

Andrew N. Hoofnagle, MD, PhD, FCAP  
Accuracy Based Testing Committee