

Educational Discussion: Accuracy Based Vitamin D

2016-B

The 2016 ABVD-B challenges are a continuation of the previously established accuracy based program for vitamin D. The samples included in the Survey were composed of pooled off-the-clot, fresh frozen serum samples 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). In collaboration with the NIH's Vitamin D Standardization Program, samples 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 samples. The minimal processing of the samples prior to distribution was vital in making samples 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-10	25.5	19.1 - 31.9	88.9/95.8	93.3
ABVD-11	35.0	26.2 - 43.8	63.3/100.0	92.5
ABVD-12	14.1	10.5 - 17.7	53.9/98.6	87.4

Passing 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 samples 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 (eg, <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 (ie, 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 (1.7 ng/mL in ABVD-10, 2.6 ng/mL in ABVD-11, and 1.4 ng/mL in ABVD-12 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. The fact that the expected concentrations for LC-MS/MS methods that do not resolve the 3-epimer (i.e., 18.5 ng/mL, 36.8 ng/mL, and 14.8 ng/mL for ABVD-10, ABVD-11, and ABVD-12, respectively) are very close to the observed mean concentrations in the Survey suggests that most of the LC-MS/MS assays do not resolve the 3-epimer. The biases observed for 25-OH vitamin D2 are most likely due to calibration.

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 (eg, 24,25-dihydroxyvitamin D3) 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 Roche instrument procedures reported significantly higher concentrations in one of the samples that contained predominantly 25-OH-D3 (ABVD-11), yet reported nearly accurate results for ABVD-12. The Roche results reported for ABVD-10 were lower than the target, which is consistent with the under-recovery of 25-OH-D2 in these assay systems, which has been seen in previous Surveys. In a previous Survey that used ABVD-12, the Vitros measurement system also reported higher than expected total 25-OH-D results, consistent with sample-specific interferences, although calibration could also be an issue. In addition, the large number of laboratories reporting results with the Siemens Centaur measurement system that fell outside the 25% acceptable range for ABVD-12 seems to be related to the high variability across sites at this low concentration (CV = 20.5%).

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.

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