COLLEGE of AMERICAN PATHOLOGISTS

Educational Discussion: 25-OH Vitamin D

2017-B Accuracy Based Vitamin D (ABVD)

The 2017 ABVD-B 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 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 Participant Summary 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.

Grading criteria for this Survey remains unchanged: for total 25-OH Vitamin D, acceptable performance requires a value within 25% or 5 ng/mL, whichever is greater, of the CDC reference value:

Total 25-OH Vitamin D (ng/mL)				
Specimen	CDC Target	Acceptable Range	Method-specific % Pass Rate (Low/High)	All Methods % Pass Rate
ABVD-04	8.75	3.7 – 13.8	85.0 / 100.0	96.3
ABVD-05	22.29	16.7 – 27.9	73.4 / 100.0	84.1
ABVD-06	25.10	18.8 – 31.4	77.3 / 100.0	91.9

Passing rates listed are for methods with a peer group n ≥ 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 (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 the National Institute of Standards and Technology (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 three specimens (<0.65 ng/mL in ABVD-04, 1.01 ng/mL in ABVD-05, and 1.17 ng/mL in ABVD-06 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 as compared to the CDC Reference Laboratory's established target values. 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 (e.g., 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***.

The specimen with a significant amount of 25-OH vitamin D2 (ABVD-05, 5.6 ng/mL) represented a very important challenge in this Survey. It is known that different immunoassays have variable recoveries of 25-OH vitamin D2, which is well exemplified in this instance. Importantly, this specimen had a total 25-OH vitamin D concentration of 22.3 ng/mL, which is near the cut-off for sufficiency suggested by the Institute of Medicine (20 ng/mL). Based on the results for the different assays provided by participants, this patient sample would be misclassified as deficient by 54-94% of laboratories running immunoassays (based on the results for assays run in at least 10 laboratories). For comparison, 9% of LC-MS/MS assays would classify the patient as deficient, but it must be remembered that LC-MS/MS assays have a positive bias for this sample due to the presence of the epimer and/or calibration. The importance of accurate measurement of 25-OH vitamin D2 must be evaluated by each medical center, because the prevalence of ergocalciferol therapy varies by practice.

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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