COLLEGE of AMERICAN PATHOLOGISTS

Educational Discussion: Vitamin D

2018-A Accuracy-Based Vitamin D (ABVD)

The 2018 ABVD-A 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.

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, or 5 ng/mL, whichever is greater (Table 2). Passing rates listed are for methods with a peer group n > 10. For this Survey, all results were above 20 ng/mL, so the acceptable range was established using +/- 25%.

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), and 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. The importance of the epimer for most LC-MS/MS assays was illustrated by sample ABVD-07, in which the concentration of C3-epi-25(OH)D3 was approximately one-tenth the concentration of native 25(OH)D3, likely contributing to the bias observed.

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***.

The sample with a significant amount of 25-OH vitamin D2 (ABVD-08, 12.6 ng/mL) represented a vitally important challenge in this Survey. It is known that different immunoassays have variable recoveries of 25-OH vitamin D2, which is well exemplified with this sample. Importantly, this sample had a total 25-OH vitamin D concentration of 25.10 ng/mL, which is well above 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 insufficient by at least 80% of laboratories running the ABBOTT ARCHITECT i, BECKMAN UNICEL DxI, ROCHE e600 SER/E170 assays*** (based on the results for assays run in at least 10 laboratories). For comparison, 3% 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.

In addition to the tables, the data obtained for each method (with a peer group n ≥ 10) are also presented in the style of box-and-whisker plots (Fig. 1). Each method is listed individually, with the number of participants using that method in parentheses after the name of the method. The individual lines extend from the minimum to maximum difference, expressed as a percentage from the target value (the percentage is a mathematical fraction). The thicker line indicates the distribution of the middle 90% of values. The grey shaded area represents the allowable error or evaluation limit, ie, ± 25% from the target. The diamond is the median for the particular method. Outliers were excluded. The presentation allows rapid visualization of bias [how far the diamond (median) is from zero], the direction of the bias indicating under or over-recovery, imprecision (length of the line) and the laboratory failures (those that lie outside the shaded area) for each method. This new feature provides additional detailed information that should be useful to individual laboratories to assess their method and compare it to both their peers and to other methods.

Manufacturers of methods that have the means furthest from the reference value and those with the largest imprecision should improve their performance, especially those methods that consistently exhibit large bias and/or large CVs.

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 measurement procedure generally rather than with how your laboratory is running it.

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