

2013 ABVD-B PARTICIPANT SUMMARY

<u>Analyte</u>	<u>Evaluation Criteria</u>
25-OH Vitamin D, total	Accuracy-Based $\pm 25\%$
25-OH Vitamin D2	Educational Challenge
25-OH Vitamin D3	Educational Challenge

~~In the event a result is not graded, a numeric code will appear next to your result. A definition of the code will appear on the first page of your evaluation. Please see "Actions Laboratories Should Take when a PT Result is Not Graded" on page 9.~~

~~To provide a timely evaluation of your results, statistics presented in this Participant Summary reflect a minimum of 80% participant enrollment.~~

The 2013 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 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 method developed by the Centers for Disease Control and Prevention Reference Laboratory (CDC), which is traceable to the NIST and Ghent reference methods. This Reference Laboratory also participates in the Vitamin D Standardization Program coordinated by the National Institutes of Health. Recently, in collaboration with the Vitamin D Standardization Program, samples in the ABVD Survey were demonstrated to be commutable and fit-for-purpose for proficiency testing of LC-MS/MS assays and other assays 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 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.

For the three samples in this Survey, LC-MS/MS methods performed well, as they have since the inception of the ABVD program. Some laboratories did not report values with the acceptable range (within 25% of the target value), which is likely due to issues with calibration 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 NIST (i.e., SRM 972a) to confirm accurate calibration of their methods or consider the use of a chromatographic method that can resolve the epimer in the analysis. Immunoassays and protein binding assays are expected to 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 onto measurements using reference LC-MS/MS methods. Generally, vitamin D metabolites are correlated with one another and as a result, the calibration of immunoassays and protein binding assays will yield accurate results on average. However, due to scatter around the regression line, these assays will produce results that are more than 25% different than the reference methods for certain samples. This is the most likely reason that different assays performed differently for ABVD-01 (from the 2013 ABVD-A Survey) and ABVD-04 (from this Survey), even though both samples had significant concentrations of 25-OH Vitamin D2. As was observed in 2013 ABVD-A, the Roche Cobas e411/elecsys and Roche Cobas e600/e170 instruments produced results for ABVD-04 that were on average at least 25% below the reference method, further demonstrating that these assays under-recover 25-OH Vitamin D2. The Abbott Architect I and the Siemens Advia Centaur assays performed better for sample ABVD-04 than for ABVD-01, suggesting that there are sample-specific interferences that can lead to under-recovery of analyte. Conversely, the Diasorin Liaison assay produced results that were on average 25% below the target value for ABVD-04, which was not seen for the ABVD-01, suggesting a different interference for this assay that can lead to under-recovery of analyte.

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 method rather than with how your laboratory is running the assay.

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