COLLEGE of AMERICAN PATHOLOGISTS

2011 ABVD-A
PARTICIPANT SUMMARY

Discussion

This Survey is the first accuracy-based 25-Hydroxy Vitamin D (25-OH Vitamin D) Survey offered by the CAP. This analyte is one for which national guidelines for diagnosis of deficiency states and treatments are based on decision limits, and it is therefore important that assays in clinical use are accurate and consistent between laboratories.

Traditional quality control and proficiency testing materials are adequate to monitor precision and conformance with peer groups. But, because of differences between those materials and real human serum ("matrix effects"), such materials cannot be used to establish accuracy. As demonstrated previously,[1] marked differences seen between different peer groups may not be present when minimally processed human serum samples are used. In that exercise, the mean values from different peer groups on typical survey material ranged from 51 to 120 ng/mL on one sample and from 70 to 150 ng/mL on a second sample, a range of more than 2-fold in each case. In marked contrast, on the fresh frozen serum sample assayed by those same labs, the mean values ranged from 23 to 30 ng/mL.

In addition to the requirement for minimally processed human serum as a material, establishment of accuracy requires measurement of that material by a reference method. Absent that, one can only say that different methods are "harmonized" (agree with each other). So, the other exercise we undertook with this survey was to arrange for each of the sample's values to be determined by the reference method. In the table below, we summarize the values provided to us by a reference laboratory at the CDC:

	25-OH Vitamin D (ng/mL)			
Sample	Total	D2	D3	D3 epimer
ABVD-01	20.8	10.6	9.7	<0.8
ABVD-02	14.6	<1.2	13.7	<0.8
ABVD-03	32.6	7.7	24.0	1.0
ABVD-04	58.5	<1.2	51.7	6.8
ABVD-05	39.9	<1.2	37.5	2.3

Discussion

As is the case with our other Accuracy-Based Surveys, you should compare your laboratory's results to the true values above. We will also report statistics for peer groups with sufficient participants. 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 by 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 lab is running the assay.

The measurement of 25-OH vitamin D presents a number of issues that complicate the evaluation of the results. First, there are two major forms of 25-OH Vitamin D: D2 (ergocalciferol, derived from plants) and D3 (cholecalciferol, the form our bodies make naturally). These two forms are not interconvertible. Most multivitamin supplements contain 25-OH vitamin D2; most vitamin D supplements contain 25-OH vitamin D3. In order to be sure we would be able to see how well participants measured each form, we supplemented some donors with 25-OH vitamin D2 before drawing their samples (all with IRB approval and informed consent) before preparing our serum donor pools. As you can see, only samples ABVD-01 and ABVD-03 had measurable levels of 25-OH Vitamin D2. Although most LC/MS methods can distinguish 25-OH Vitamin D2 and 25-OH Vitamin D3 from each other, immunoassays typically do not. Some immunoassays measure only one form, some measure both forms on an equimolar basis, and some measure the different forms with different cross-reactivities (e.g., 100% for 25-OH Vitamin D3 versus 75% for 25-OH Vitamin D2).

Second, there is a distinct species, an epimer of 25-OH Vitamin D3, which again is measured differently by different methods. As can be seen from the data above, it is usually a small proportion of the 25-OH Vitamin D, but, when present in measurable amounts, it may account for some of the differences among methods. In future Surveys, we will ask participating laboratories whether their result includes a measurement of the epimer.

Third, the 25-OH Vitamin D guidelines themselves are a moving target. In late 2010, the Institute of Medicine published revised guidelines for target levels for 25-OH Vitamin D, which included lowering the lower limit for sufficient from 30 to 20 ng/mL as well as indicating that levels higher than 50 ng/mL "should raise concerns."[2] As stated in the report, what this new lower limit means is that the prevalence of vitamin D deficiency in the United States has been seriously overestimated. Notwithstanding this concern, a useful feature of the current Survey is that the samples span the clinically important range quite well: deficient (14.6), close to the new lower bound for sufficient (20.8), close to the former lower bound for sufficient (32.6), sufficient (39.9), and supratherapeutic (58.5).

Given all these variables (first mailing of the Survey, variable sensitivity to D2/D3, unclear inclusion of D3 epimer), we decided to use a relatively liberal grading criterion for acceptable performance: within 25% of the target value. In the table below, we summarize the acceptable ranges for Total 25-OH Vitamin D:

COLLEGE of AMERICAN PATHOLOGISTS

Discussion

Sample	Total	Acceptable Range	Overall Pass Rate
ABVD-01	20.8	15.6-26.0	83%
ABVD-02	14.6	10.9-18.3	87%
ABVD-03	32.6	24.4-40.8	88%
ABVD-04	58.5	43.8-73.2	90%
ABVD-05	39.9	29.9-49.9	92%

Even with this liberal criterion, you can see that the acceptable range crosses a decision limit only for samples ABVD-01 and ABVD-04.

Laboratories should carefully review the agreement of their data with the target values. If you know your method does not detect 25-OH Vitamin D2 at all, you can compare your results to just the 25-OH Vitamin D3 values. Or, if you use an immunoassay with 100%/75% cross-reactivity to the D3/D2 forms, you can correct the expected concentrations for samples ABVD-01 and ABVD-03. And, if your 25-OH D3 results include the D3 epimer, you can add that value back into the expected value for 25-OH vitamin D3. (According to the CDC, NIST recommends not including it, so the CDC follows that recommendation, and the Table above lists them separately.)

We thank you for your participation and hope you find these results as interesting and informative as we did. We look forward to the next mailing of the 25-OH Vitamin D Accuracy Based Survey (to be mailed in 2012).

Gary L. Horowitz, MD
Anthony A. Killeen, MD, PhD

Referrences

1. Participant Summary Report from Survey 2009 Y-A of the College of American Pathologists. Available at http://www.cap.org/apps/docs/committees/chemistry/measurements_25_OH_vitamin_d.pdf
2. Ross AC, Manson JE, Abrams SA, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. J Clin Endocrinol Metab. 2011; 96:53–58