Letters to the Editor

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Influence of Vitamin D2 on Accuracy of 4 Commercial Total 25-
Hydroxyvitamin D Assays

To the Editor:

25-Hydroxyvitamin D (25-OH-D) is the most appropriate marker
for monitoring vitamin D storage status. The 2 major forms
of 25-OH-D [25-OH-D2 (D2) and 25-OH-D3 (D3)], differ slightly
in molecular structures but have essentially the same physiological
activity. Therefore, both D2 and D3 should be measured to assess vitami
D status. At our laboratory, 42% of patient samples submitted
for vitamin D testing contain D2. Recognizing the ability of an assay
to quantify both the D3 and D2 forms equally is therefore crucial.

The analytical performance of several total 25-OH-D assays
approved by the US Food and Drug Administration is well docu-
mented (1); however, information is limited on how D2 affects assay
accuracies. Le Goff et al. (2) demonstrated the inconsistency be-
tween several assays in their cross-reactivities toward D2. The size
of their study was small, however, and the range of D2 percentages (D2%)
was unknown. We used a larger sample size than previous studies
and a D2% interval of 1.3%–91.2% [3.4–95.1 ng/mL (8.5–238 nmol/
L)] and evaluated the influence of D2% on the accuracies of the fol-
lowing assays: DiaSorin Liaison, Abbott Architect, Roche Cobas,
and Siemens Centaur. We developed and validated a liquid
chromatography–tandem mass spectrometry (LC-MS/MS) assay as
the reference method.

Samples were prepared for the
LC-MS/MS assay by mixing with
internal standard (D1-d6/D2-d2),
ZnSO4 solution, and methanol;
liquid–liquid extraction (hexane);
drying; and reconstitution. The
liquid chromatography method was established with a Phenome-
nex 2.6-μm Kinex C18 column
(2.1 × 50 mm) at 35 °C and a flow
rate of 0.35 mL/min. Mobile phases
A and B were deionized water and
LC-MS-grade methanol, respec-
tively, both containing 1 mL/L for-
ic acid and 2 mmol/L ammoni-
um acetate. The linear gradient of
the mobile phase was 60% to
100% B in 3.5 min, and the total
length of the liquid chromatogra-
phy run was 8 min. A Waters
Micromass Quattro Micro mass
spectrometer, operated with posi-
tive electrospray ionization, was
used to acquire chromatograms
with the following ion transi-
tions: D2, m/z 401.0 → 159.1; D3,
D3–D6, m/z 413.0 → 355.2; D3–D6,
D2, m/z 407.0 → 159.1; and D2–D6,
D2, m/z 419.0 → 355.2.

The between-day CVs were
between 5.1% and 12% at D2, D3,
and total D concentrations of
12.9–143 ng/mL (32.2–358 nmol/
L). The matrix effect was negli-
gible. The linearity intervals for D2,
D3, and total D were 3.0–200
ng/mL (7.5–500 nmol/L), 1.0–150
ng/mL (2.5–375 nmol/L), and 3.0–
350 ng/mL (7.5–875 nmol/L), re-
spectively. The recoveries were
83.8%–109.2% (mean, 95.9%).
The lower limits of quantification
for D1 and D2 were 3.0 ng/mL (7.5
nmol/L) and 1.0 ng/mL (2.5 nmol/
L), respectively. We tested the 2011
College of American Pathologists
proficiency testing set ABVD-
01–05 with the assay, and all re-
results were within ±25% of target
values (mean bias, −1.2%). Inter-
ference by 3-epi-25-OH-D3 was
negligible, because our study in-
cluded only samples from adults
(21–97 years) (3).

We randomly chose samples
from 149 adult patients submitted
for 25-OH-D testing by the Abbott
Architect assay. All samples were
tested by the LC-MS/MS assay (63
samples with D1%); and subsets of these
samples were aliquoted for the fol-
lowing assays: 75 samples (31 with
D2) by the Liaison assay, 64 sam-

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ple with D3 only vs. (b) all
samples. The regression equations
and the coefficients of determina-
tion (r2) obtained for each scenario
are shown in Fig. 1A.

For the Liaison assay, the 2
scenarios yielded similar coeffi-
cients of determination; however,
these values were different for all of
the other assays. The regression
slopes and r2 values of the Archi-
tect and Cobas assays both de-
Fig. 1. Correlation of manufacturers’ assays for total 25-OH-D concentration in 2 scenarios (A) and bias relative to the LC-MS/MS results at different D2% values (B).

(A), Solid circles are D3 only and crosses are all other samples. Solid line indicates the diagonal. Regression lines are dashed for samples with D3 only and dotted for all samples. (B), Only D2-containing samples are plotted. The gray line indicates 0% bias relative to LC-MS/MS results; the dashed line indicates the trend of the bias change for each of the assays. D2 cross-reactivity is presented as the mean (first to third quartiles) and is calculated as follows: D2 cross-reactivity = \((\text{assay total D}) / \text{LC-MS/MS total D}) \times 100\%\). The second term in the numerator is obtained from regression equations with D3 only samples and provides an estimate of D3 in a commercial assay. The denominator term represents a D2 estimate by the total assay with 100% D2 cross-reactivity.
creased when D$_2$-containing samples were included. For the Centaur assay, on the other hand, the regression slope increased, and the $r^2$ decreased. The mean (SD) changes in bias from scenario (a) to (b) for the 4 assays are as follows: Liaison assay, $-17.4%$ (6.0%) to $-19.2%$ (4.6%); Architect assay, 0.9% (5.2%) to $-7.8%$ (4.2%); Cobas assay, $-11.4%$ (11.9%) to $-15.5%$ (7.0%); Centaur assay, $-27.7%$ (7.0%) to $-17.0%$ (8.0%).

The effect of D$_2$% on assay bias is illustrated in Fig. 1B. The Liaison assay had an insignificant bias change as D$_2$% increased. The architect and Cobas assays showed an increasingly negative bias with increasing D$_2$%, whereas the negative bias of the Centaur assay decreased and changed to a positive bias.

We calculated D$_3$ cross-reactivity normalized to D$_2$ reactivity (see Fig. 1 legend). The values for each assay in Fig. 1B are displayed as the mean (first to third quartiles) and are consistent with the change in the assay’s bias with D$_2$%. Although cross-reactivities are defined differently from Le Goff et al. (2) and the manufacturers’ claims, our results showed the same order of relative D$_2$ cross-reactivity: Centaur > Liaison > Cobas > Architect.

Although D$_3$ is the preferred supplement form for treating vitamin D deficiency (4, 5), we have observed a high prevalence of patient samples containing substantial amounts of D$_2$, and we have demonstrated the different effects of D$_2$ content on the accuracies of commercial assays. Therefore, correctly interpreting total 25-OH-D results and monitoring patient compliance requires that vitamin D status, type of supplementation, and measurement method be considered.

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References


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Letters to the Editor

Dried Blood Spot Quality Control Materials for Newborn Screening to Detect Lysosomal Storage Disorders

To the Editor:

It is with great interest that we read the recent report by Spacil et al. on a high-throughput assay to detect 9 lysosomal enzymes from dried blood spots (DBS) collected by newborn screening programs (1). Newborn screening activities to detect lysosomal storage disorders (NBS-LSD) have generated a great deal of discussion worldwide. The availability of tandem mass spectrometry–based assays suitable for high-throughput population screening and of US Food and Drug Administration–registered reagents for use in these assays led to an ongoing worldwide conversation about optimizing NBS-LSD assays. Recognizing the lack of DBS QC for NBS-LSD, the Newborn Screening Translation Research Initiative at the CDC developed large-scale methods to produce DBS that emulate normal and deficient lysosomal enzyme activities. The preparation and evaluation of these materials was originally reported in this journal (2). These DBS-QC have been in use globally for over 5 years, and they were a critical element of the assay validation reported by Spacil et al. We want to be sure that Clinical Chemistry readers can lo-