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Performance evaluation of Siemens ADVIA Centaur and Roche MODULAR Analytics E170 Total 25-OH Vitamin D assays

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ABSTRACT

Objectives: To evaluate the newly developed Roche MODULAR Analytics E170 Total Vitamin D and the Siemens ADVIA Centaur® Vitamin D Total assays.

Materials and Methods: Assays were evaluated using the Clinical and Laboratory Standards Institute protocols. Split patient samples were compared with LC-MS/MS and DiaSorin LIAISON assays ($n = 79$ including 15 specimens with detectable endogenous 25-OH vitamin D_2). Assay accuracy was also evaluated using the Vitamin D External Quality Assessment Scheme (DEQAS) samples.

Results: The ADVIA Centaur and E170 assays demonstrated maximum total CVs of 14.1% and 5.9%, respectively. Both showed excellent linearity ($R^2 > 0.99$). The ADVIA Centaur assay demonstrated interference with bilirubin at 800 μmol/L, hemolysis at 1.25 g/L, and triglycerides at 2.8 mmol/L. Compared to LC-MS/MS, the ADVIA Centaur assay demonstrated a R^2 value of 0.893, average bias of −8.8%; the E170 assay an R^2 value of 0.872, average bias of 14.3% with underestimation of 25-OH vitamin D_2. Compared to the LIAISON assay, the ADVIA Centaur assay demonstrated an R^2 value of 0.781, average bias of −17.3%; the E170 assay an R^2 value of 0.823, average bias of 11.4%. The ADVIA Centaur and E170 assays demonstrated a biases of <20% in 10/10 and 8/10 DEQAS samples, respectively.

Conclusions: The ADVIA Centaur and E170 vitamin D assays demonstrated acceptable linearity, imprecision, and accuracy. The E170 assay demonstrated consistent underestimation of 25-OH vitamin D_2 levels. Compared with LC-MS/MS, the ADVIA Centaur assay demonstrated a higher R^2 value and a smaller average bias than the E170 assay.

Introduction

Vitamin D testing (25-OH VitD) has increased 20-fold in our laboratory over the past 5 years. Testing has been increasing because several studies have shown that large segments of the population have inadequate levels of vitamin D (deficient or insufficient levels) [1–6], and other studies have demonstrated an association between vitamin D levels and the development of certain types of cancers, and risk for cardiovascular disease [2–8]. Adequate levels of vitamin D are considered to be ≥75 nmol/L (30 ng/mL), insufficient levels 25–74 nmol/L (10 ng/mL to 29 ng/mLl), and deficient levels <25 nmol/L (10 ng/mL) [7–9]. Vitamin D plays an essential role in maintaining good bone health; individuals who are deficient for vitamin D are at risk for fractures, osteomalacia, and rickets [6,9].

In Canada, the US and other countries, the widely available pharmaceutical preparation is vitamin D_2. Total 25-OH vitamin D assays (detect and measure 25-OH D_2 and 25-OH D_3) are important because they allow for an accurate assessment of individuals' vitamin D status [7,10,11]. Vitamin D_2 and vitamin D_3 supplements are typically prescribed to individuals who are deficient. An assay that is unable to detect all of or only a portion of the vitamin D_2 in circulation is not appropriate for these individuals as it will not be able to provide an accurate assessment of their status. The consensus is that a total vitamin D assay is preferred in routine vitamin D testing and assays like LC-MS/MS that measure the analytes separately should provide clinicians with a total value [7,11].

Vitamin D is not an easy analyte to measure, primarily because it is hydrophobic and has a high affinity for vitamin D binding protein [10,12,13]. All assays, independent of the method, start with a separation of vitamin D from its binding protein. Assays differ in how they accomplish this step, and also vary in how they detect and measure the displaced vitamin D, thus resulting in a great deal of variation

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among vitamin D assays [12]. Studies that compared several different assays (HPLC, automated immunoassay, RIA, and protein binding assays) to LC-MS/MS demonstrated Passing–Bablok slopes of 0.62 to 1 [10,12–14]. This variation, however, has been improving and is expected to improve more with the release of commutable reference materials and a standard reference procedure. A Vitamin D Standardization Program has been established by the National Institutes of Health Office of Dietary Supplements (NIH ODS), Centers for Disease Control and Prevention National Center for Environmental Health (CDC) and National Institute for Standards and Technology (NIST) with reference laboratories at NIST and the University of Ghent [11,15]. A recent comparison of this reference method and routine LC-MS/MS methods showed that the reference method results were on average 11.2% lower than those given by routine LC-MS/MS methods [13]. This program will standardize measurement results from national survey laboratories as well as clinical laboratories and assay manufacturers by developing a reference method that should help address the variations among assays [16]. The initial assay performance criteria for this standardization program are ≤10% imprecision and ≤5% bias compared to the reference values [16]. New human serum based commutable reference materials should help as well [11,15].

Laboratories are challenged to provide reliable total vitamin D results in a timely, cost-effective manner. Our goal was to choose an automated total 25-OH vitamin D assay based on a performance evaluation that compared the Roche MODULAR Analytics E170 Total Vitamin D (E170) assay and the Siemens ADVIA Centaur® Vitamin D Total (ADVIA Centaur) assay to LC-MS/MS. Both assays were also compared to the DiaSorin LIAISON 25 OH Vitamin D TOTAL (DiaSorin LIAISON) assay. We used the results from the evaluation and the criteria delineated by Stockl et al. [17] for a routine measurement procedure to guide our decision.

Methods

The ADVIA Centaur (lot# 002) and E170 (lot#167216) vitamin D assays were evaluated using CLSI evaluation protocols for testing precision (EP5) [9,18], accuracy (EP15) [19], linearity (EP6) [20], interference (EP7) [21], and matrix effects (EP14) [22], as well as protocols for evaluating quantitative and qualitative methods (EP10, EP12) [23,24], for estimating bias (EP9) [25], and estimating total analytical error (EP21) [26]. The method comparison study also included the DiaSorin LIAISON and LC-MS/MS (reference method) assays. In addition, 10 DEQAS samples were used to evaluate ADVIA Centaur and Roche E170 assay accuracy.

Assays

ADVIA Centaur® Vitamin D Total

The ADVIA Centaur Vitamin D Total assay (Table 1) is an 18-min automated direct competitive chemiluminescent immunoassay that detects 25-OH vitamins D_2 and D_3 in serum or plasma. This assay uses a proprietary releasing reagent and a monoclonal antibody. This assay is standardized against LC-MS/MS. The assay range is 9.3 nmol/L to 375 nmol/L.

Roche MODULAR Analytics E170 Total Vitamin D

The Roche MODULAR Analytics E170 Total Vitamin D assay (Table 1) is a 27-min automated direct competitive electrochemiluminescence immunoassay that detects 25-OH vitamins D_2 and D_3 in serum or plasma. This assay is standardized against LC-MS/MS. The assay range is 7.50 nmol/L to 175 nmol/L.

DiaSorin LIAISON 25 OH Vitamin D TOTAL

The DiaSorin LIAISON 25 OH Vitamin D TOTAL assay is an automated direct competitive chemiluminescent immunoassay that detects 25-OH vitamins D_2 and D_3 and other hydroxylated metabolites in serum or plasma. The assay range is 10 nmol/L to 375 nmol/L.

Table 1
Selected assay characteristics from the package inserts of the ADVIA Centaur and E170 vitamin D assays.

	Siemens Centaur	Roche Modular
Assay format	Direct, competitive, CLIA	Direct, competitive, ECLIA
Sample volume	20 μL	15 μL
Sample type	Serum and plasma	Serum and plasma
Assay time	18 min	27 min
Analytical sensitivity (LoD)	8.0 nmol/L	7.5 nmol/L
Analytical sensitivity (LoQ)	8.8 nmol/L	22.5 nmol/L
Assay range	9.3–375 nmol/L	7.5–175 nmol/L
Analytical specificity	% Cross-reactivity	% Cross-reactivity
Vitamin D_3	0.3	5.0
Vitamin D_2	0.5	6.0
25(OH)D_3	100.7	98.0
25(OH)D_2	104.5	81.0
1,25(OH)$_2D_3$	1.0	5.0
1,25(OH)$_2D_2$	4.0	6.0
24,25(OH)$_2D_3$	na	121.0
Paricacitol	0.1	na
C_3-epimer of 25(OH)D_3	1.1	93.0
Traceability	Traceable to LC-MS/MS	Traceable to LC-MS/MS

Liquid chromatography–tandem mass spectrometry with TurboFlow Online Extraction (LC-MS/MS)

One hundred microliters of serum sample, calibrator or quality control was aliquoted into a microcentrifuge tube. 25 μL of internal standard (250 nM, D6-25-OH D_3, Medical Isotopes Inc., Pelham, NH, USA) was added to each sample, followed by vortexing. Analytes were extracted by protein crash with 175 μL acetonitrile. Each solution was vortex-mixed for 1 min and centrifuged for 10 min. The supernatant was transferred into an autosampler vial. 20 μL of each supernatant solution were injected into the TLX-2-MS/MS for a 5.6-min analysis.

The chromatographic separation of 25-OH D_2 and 25-OH D_3 was carried out using a TLX-2 Turbo Flow system (Thermo Fisher Scientific, Franklin, MA). The XL C18-P, 0.5 × 50 mm, (Thermo Fisher Scientific, Franklin, MA) TurboFlow column was chosen for online extraction and the Hypersil gold C18, 3.0 × 50 mm, 3 μm (Thermo Fisher Scientific, Franklin, MA) LC column was chosen for the final separation prior to MS detection. The multisolvent pumps were programmed with Aria software, version 1.6, to automatically apply different mobile phase conditions to both the extraction and separation columns. In the first chromatographic dimension the sample was loaded onto the extraction column where small molecules, including 25-OH D_3 and D_2 were retained. Matrix contaminants were removed by flushing the TurboFlow column at a high flow. The analytes were then eluted onto the analytical column for separation. MS detection was carried out with an API 5000 (AB SCIEX, Toronto, Canada) mass spectrometer operating in multiple reaction monitoring (MRM) positive ion mode using following transitions: 401.4/383.4 and 401.4/365.4 for 25-OH D_3, 413.4/395.4 and 395.4/377.4 for 25-hydroxyvitamin D2; and 407.4/389.4 for the internal standard, D6-25-OH D_3. MRM acquisition was controlled by Analyst 1.4.2 software (AB SCIEX). Vitamin D metabolites and internal standards eluted at approximately 3.3 min from the LC column. In a typical 5.6 minute run, the mass spectrometer was alternating data collection while the peaks of interest were eluting from both LC systems. In the multiplexing mode, MS data were acquired for only 45 s which greatly reduced the amount of mobile phase entering the instrument. The instrument response was obtained for six calibration standards (0–250 nmol/L). Ratio of

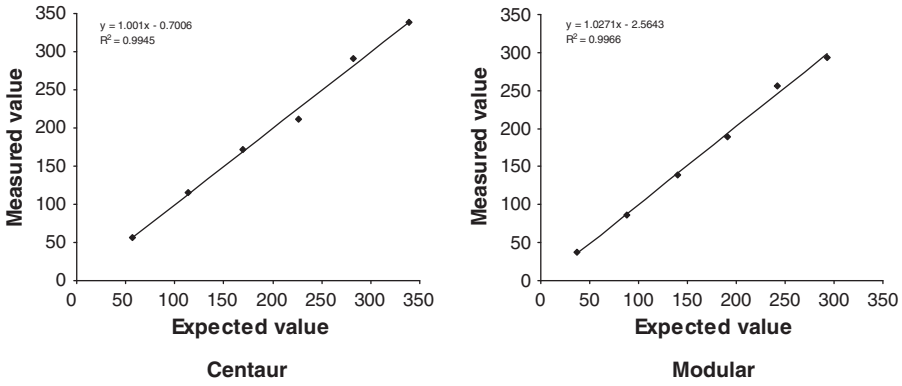

Fig. 1. Linearity of the ADVIA Centaur and Modular E170 Total Vitamin D assays.

analyte peak area/internal standard peak area was plotted against the analyte concentration and the points were fitted using linear regression analysis. Serum 25-OH D_2 and 25-OH D_3 concentrations were obtained from the regression equation.

The LC-MS/MS assay was linear between 10 and 250 nmol/L for both 25-OH D_3 and 25-OH D_2 and showed good linearity, with R^2 greater than 0.999 for both analytes. The total CVs for different concentration levels of both analytes were between 3.6% and 14.5% for levels between 24 and 134 nmol/L for 25-OH D_3 and between 12 and 99 nmol/L for 25-OH D_2. The mean relative recoveries were 102.4% and 106.6% for 25-OH D_3 and D_2, respectively. The limit of quantification for both analytes based on a functional sensitivity of 20% CV was 3.5 nmol/L, while the LODs for 25-OH D_3 and 25-OH D_2 were 0.12 nmol/L and 0.15 nmol/L, respectively. The LC-MS/MS assay was calibrated using the NIST SRM 972.

Sample collection

Remnant anonymous samples stored as frozen aliquots and previously collected at Dr. Everett Chalmers Regional Hospital (Fredericton, NB, Canada) and Toronto General Hospital (Toronto, ON, Canada) were used. Seventy-nine samples across the assays' measuring ranges, among them 15 with detectable 25-OH vitamin D_2 (endogenous) were evaluated.

Assay linearity

Linearity was evaluated for the ADVIA Centaur and E170 assays using six dilutions. Dilutions were run in duplicate in a single run on a single ADVIA Centaur system and a single E170 Modular system.

Assay imprecision

Within-run CVs were determined for the ADVIA Centaur and E170 assays using two to three sample pools of known concentration (high, medium, low); each sample was run in replicates of 10–15 on a single day. Total CVs were determined using the same two to three sample pools run in singlicate for 20 days.

Sample carry-over

Although carry-over studies more typically would involve looking for analyte in blank samples, sample carry-over was evaluated for the ADVIA Centaur and E170 assays using three replicates of a high concentration sample (sample a), immediately followed by three replicates of a low concentration sample (sample b). Carry-over (k) was calculated using the equation $(b_1 - b_3)/(a_3 - b_3)$, as described previously [27–29]. The sample carryover evaluations were performed on different occasions using different sample pools. This sample carry-over equation presumes that any differential between each of the three high samples and each of the three low samples should be attributable only to with-in run variability in the absence of carry-over. If there is carry-over, however, samples a_2 and a_3 should be somewhat greater than expected, with the relative increase in sample a_3 being potentially larger than that of a_2. Likewise, if there is carry-over, sample b_1 should be greater than expected, as should samples b_2 and b_3, thus it's only necessary to determine the difference between the 1st and 3rd of each series to estimate carry-over (k).

Interference

Interference from bilirubin, lipemia and hemolysis was evaluated for the ADVIA Centaur and E170 assays by spiking high- and low-

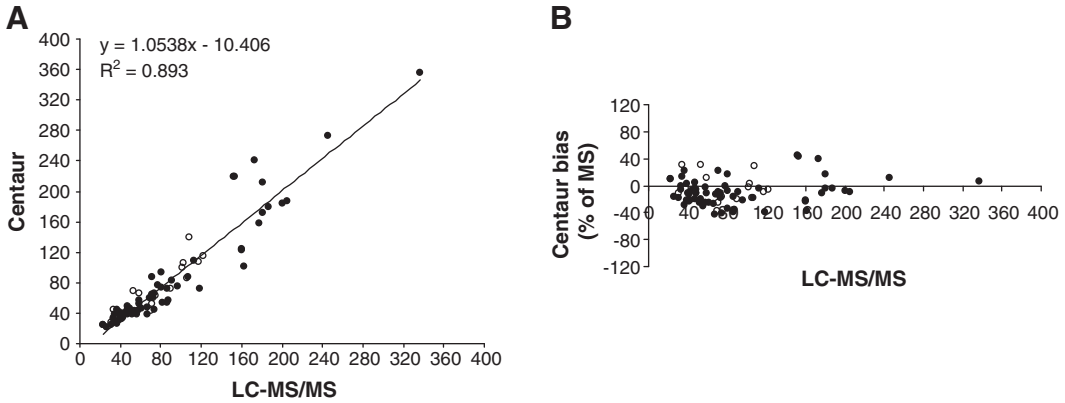

Fig. 2. Linear regression (A) and Bland–Altman analysis (B) for the ADVIA Centaur assay versus the LC-MS/MS assay. ○ dots represent endogenous 25-OH D_2 containing samples ($n = 15$), ● dots represent samples without detectable 25-OH D_2 ($n = 64$).

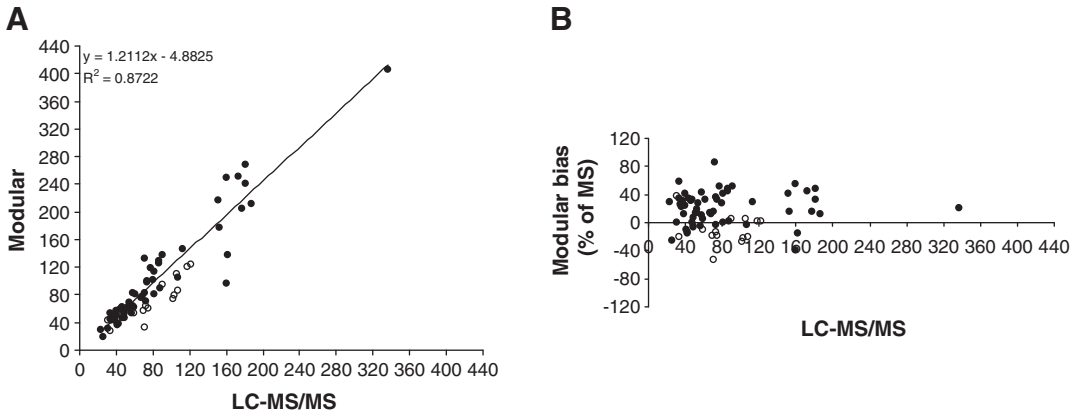

Fig. 3. Linear regression (A) and Bland–Altman analysis (B) for the E170 assay versus the LC/MS/MS assay. ○ dots represent endogenous 25-OH D_2 containing samples ($n = 15$), ● dots represent samples without detectable 25-OH D_2 ($n = 64$).

concentration patient sample pools with high to low levels of bilirubin or triglycerides, or high to low levels of erythrocyte hemolysate. Observed vs. target recovery of 25-OH vitamin D in the presence of each interferent was calculated. Recovery was considered acceptable if the observed value was no more than ± 15% of the target value.

Method comparisons

The ADVIA Centaur, E170 Modular, LIAISON assays were run according to the manufacturer's specifications. LC-MS/MS was performed as described. Seventy-nine patient samples within the assays' measuring ranges which included 15 samples with detectable 25-OH vitamin D_2 were used. Samples for the correlation studies and comparison to LC-MS/MS were run in singlicate for a single run.

Assay accuracy

Ten Vitamin D External Quality Assessment Scheme (DEQAS) (#381 to 390) samples were used and run in triplicates to evaluate ADVIA Centaur and Roche E170 assay accuracy.

Statistical methods

Statistical analysis was carried out using Microsoft Excel. The best fit line by linear regression was used to evaluate assay linearity. Regression analysis was also used to evaluate method correlations, and Bland–Altman plots were constructed to assess systematic bias between methods.

Results

Assay linearity

Both the ADVIA Centaur and E170 assays demonstrated good linearity in wide range (up to 339 nmol/L for ADVIA Centaur, and up to 293 nmol/L for E170 assays), with R^2 values of 0.9945 and 0.9966, respectively (Fig. 1).

Assay imprecision

The ADVIA Centaur assay demonstrated within-run and total imprecisions of 6.4% and 14.1% at 52.6 nmol/L, and 4.2% and 7.4% at 225 nmol/L; There were two significant outlier results for the ADVIA Centaur total CV at 52.6 nmol/L that increased the CV at this concentration. The E170 assay within-run and total CVs of 2.6% and 5.9% at 41.3 nmol/L, 1.7% and 1.9% at 129.5 nmol/L, and 1.5% and 2.8% at 204.6 nmol/L.

Sample carryover

Sample carry-over was evaluated for the ADVIA Centaur (this platform uses disposable pipette tips to obviate sample carryover) and E170 assays using 3 replicates of a high concentration sample pool (sample a: ADVIA Centaur: 218.7 nmol/L, 240.5 nmol/L, 233.1 nmol/L; E170: 126 nmol/L,125.5 nmol/L, 122.1 nmol/L), immediately followed by 3 replicates of a low concentration sample pool (sample b: ADVIA Centaur: 58.3 nmol/L, 51.5 nmol/L, 57.5 nmol/L; E170: 7.5 nmol/L, 7.5 nmol/L, 7.5 nmol/L). Carry-over (k) for the ADVIA Centaur was

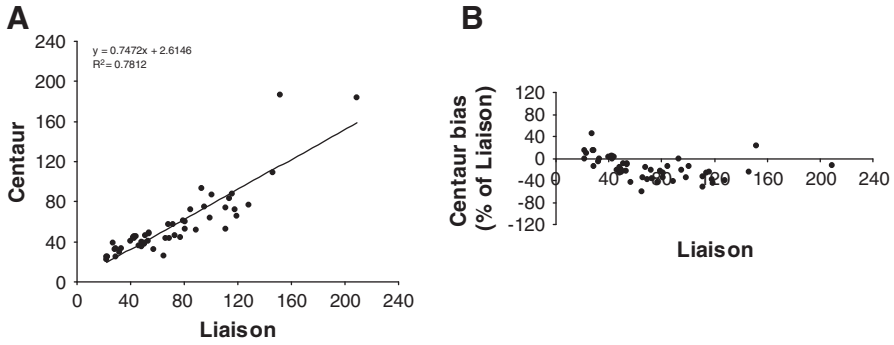

Fig. 4. Linear regression (A) and Bland–Altman analysis (B) for the ADVIA Centaur assay versus the LIAISON assay ($n = 50$).

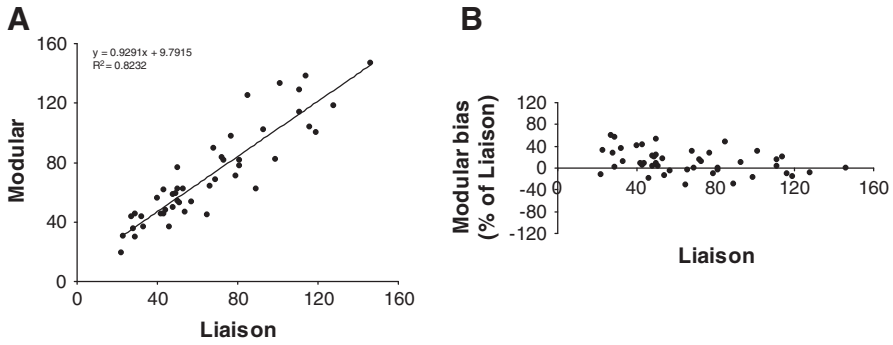

Fig. 5. Linear regression (A) and Bland–Altman analysis (B) for the E170 assay versus the LIAISON assay ($n = 50$).

0.0046 and for the E170 Modular 0.00, indicating that sample carryover is negligible.

Interference

The ADVIA Centaur assay demonstrated interference with bilirubin at 800 μmol/L, hemolysis at 1.25 g/L, and triglycerides at 2.8 mmol/L. The E170 assay did not demonstrate interferences with bilirubin at 800 μmol/L, hemolysis at 5 g/L, and triglycerides at 11.3 mmol/L (Supplemental Tables 1, 2).

Assay accuracy

The ADVIA Centaur results differed from the assigned DEQAS survey sample values (all method means) by 18.5% to −13.7%, and the E170 assay by 43.4% to −1.6%. The ADVIA Centaur assay demonstrated a bias of less that 20% in 10/10 samples versus the E170 assay in 8/10 samples (Supplemental Tables 3, 4).

Method comparisons

In comparison to LC-MS/MS, the ADVIA Centaur assay demonstrated an R^2 value of 0.893 (Fig. 2A), with an average bias of −8.8% (Fig. 2B), and the E170 assay an R^2 value of 0.872 (Fig. 3A), with an average bias of 14.3% (Fig. 3B). Regression and Bland–Altman analyses demonstrated underestimation of 25-OH vitamin D_2-containing samples for the E170 assay (Fig. 3B).

Compared to the DiaSorin LIAISON assay, the ADVIA Centaur assay demonstrated an R^2 value of 0.781, with an average bias of −17.3% (Fig. 4), and the E170 assay an R^2 value of 0.823, with an average bias of 11.4% (Fig. 5).

Compared to LC-MS/MS, the LIAISON assay demonstrated an R^2 value of 0.7984 with an average bias of 9.2% (Supplemental Fig. 1).

Discussion

In this study, the Siemens ADVIA Centaur and Roche E170 vitamin D assays demonstrated acceptable linearity (~0.99) and imprecision. The ADVIA Centaur and E170 assays both met the DEQAS accuracy criteria for a bias of <20% in 80% tested samples, but the ADVIA Centaur met these criteria for all DEQAS samples that were tested. Using LC-MS/MS as the reference method, the ADVIA Centaur assay demonstrated a higher R^2 value (0.893) and a smaller average bias (−8.8%) than the E170 assay ($R^2 = 0.872$, average bias = 14.3%). Furthermore, the E170 assay demonstrated consistent underestimation of 25-OH D_2 levels. In addition, the E170 assay demonstrated higher precision and did not show interference at the concentrations tested for hemolysis, bilirubin, and lipids.

Our study demonstrated a positive bias of LIAISON results compared to LC-MS/MS results, as was also demonstrated in another study by Becker et al. [30]. The results of our study were also consistent with the findings of Moon et al. [31] which showed that compared to LC-MS/MS the ADVIA Centaur, E170 and LIAISION assays demonstrated acceptable correlations. Consistent with the findings by Moon et al., we also demonstrated a negative bias for the ADVIA Centaur and positive bias for the E170 assays compared to LC-MS/MS. However, while our study demonstrated a positive bias for the LIAISON assay compared to LC-MS/MS, the Moon et al. [31] study demonstrated a negative bias. The current E170 assay that is able to detect both 25-OH D_2 and 25-OH D_3 is a great improvement over the previous version which only detected 25-OH D_3. The 25-OH D_3 only assay is no longer commercially available and a few studies were shown to consistently underestimate total 25-OH D levels thus resulting in individuals being misclassified as deficient [32,33].

Vitamin D testing continues to be a challenge for the clinical laboratory, which is expected to provide reliable results in a timely manner for this high volume assay. The ideal vitamin D assay is one that is precise, accurate, and timely; most available assays could benefit from improvements in these desired traits. Vitamin D is not an easy analyte to measure. Some key issues for immunoassays include lot-to-lot variation, human antianimal antibody interferences, interferences from other hydroxylated vitamin D metabolites, and the ability to separate 25-OH D from its binding protein [34]. There is a great deal of variety among 25-OH D assays because of the differing techniques employed for separating 25-OH D from its binding protein, its detection, and its measurement [34]. The Vitamin D Standardization program (collaboration of the NIH, the CDC, University of Ghent, and the NIST) has developed a reference method and materials will likely facilitate better agreement among vitamin D assays [11,15]. It is expected that the manufacturers of vitamin D assays will either standardize to or be traceable to this reference method.

In lieu of the availability of the Vitamin D Standardization reference method and materials, we relied on performance goals for CV and bias detailed in the laboratory data model (CV<15%, bias <10%) and expert opinion DEQAS model (CV<22%, bias<10%) summarized by Stockl et al. [17] for a routine measurement procedure. The ADVIA Centaur assay met the criteria for both models, while the E170 assay demonstrated excellent precision but a bit higher than expected bias. We chose to implement the ADVIA Centaur assay for routine vitamin D testing in our laboratory. Meanwhile, our study suggested that there are still lots of room for the ADVIA Centaur assay to improve its analytical precision and to reduce interference by hemolysis, bilirubin, and lipid.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.clinbiochem.2012.06.002.

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