Current Status of Clinical 25(OH)D Measurement

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ABSTRACT

Measurement of circulating 25-hydroxyvitamin D (25(OH)D) is accepted as the clinical indicator of vitamin D status. However, the clinical measurement of 25(OH)D has been problematic. It might be assumed that the recent widespread clinical availability of liquid chromatography tandem mass spectroscopy (LCMSMS) and high-pressure liquid chromatography (HPLC) technologies has improved 25(OH)D assay performance, thereby improving agreement between measurements obtained at different clinical laboratories. However, a review of recent DEQAS (vitamin D External Quality Assessment Scheme) data revealed substantial variability in individual 25(OH)D measurements between laboratories using LCMSMS and HPLC, despite good agreement of mean values. We hypothesized that this between-laboratory variability reflects differences in assay calibration. To evaluate the current clinical status of 25(OH)D measurement in the U.S., and investigate the possibility that simple assay calibration would improve between-laboratory agreement, 15 serum specimens and one "calibrator," selected to have a 25(OH)D value near 30 ng/ml, were analyzed in four clinical laboratories.

Serum specimens were obtained from 15 volunteers (age 20-60 years), aliquots were prepared and frozen at -80°C and sent as clinical specimens for 25(OH)D measurement to four clinical laboratories, (labs A-D). The methodology used for 25(OH)D measurement was as follows: Lab A, HPLC; Labs B and C, LCMSMS; Lab D, RIA. Based upon self-reported inter-assay %CV, results from lab A were assigned as the reference to which the other labs were compared using linear regression and Bland-Altman analyses. For this report, vitamin D deficiency and insufficiency were defined respectively as < 15 ng/ml and < 30 ng/ml.

Overall, good diagnostic agreement was present with 73% of volunteers classified in the same category in all four labs. However, in a substantial minority, classification of vitamin D status was dependent on the lab in which their specimen was measured with 20% changing between insufficent/adequate and 7% between deficient/insufficient. Moreover, good to excellent correlation was observed for 25(OH)D measurement between lab A and labs B-D; R^2 = 0.99, 0.81 and 0.95 respectively. Modest between-lab variation was noted; the mean bias ranged from +2.9 ng/ml to +5.1 ng/ml. Consistent with a systematic offset, each value in lab B was higher than that in lab A (mean 8.7%, range 0.3% to 19.6%) and 89% of values from labs B-D were higher than lab A. As it could be hypothesized that a standard calibrator would reduce this systematic bias, the results from labs B-D were "corrected" by measurement of a sample for which lab A obtained a value of 28.1 ng/ml. This adjustment reduced mean between lab bias for labs B and D.

In conclusion, the current clinical measurement of 25(OH)D in these four laboratories results in the majority of these individuals vitamin D status being classified similarly. Moreover, between lab agreement overall is good to excellent. Use of even a single calibrator will improve, but not resolve, between-lab variability. Further measures to improve between clinical laboratory agreement appear likely to require use of more than one calibrator and measures to enhance 25(OH)D assay precision.

INTRODUCTION

Measurement of circulating 25(OH)D is accepted as the best clinical indicator of an individual's vitamin D status. As such, 25(OH)D measurement has become widely utilized by clinicians with the diagnosis of vitamin D insufficiency or deficiency being made, and treatments prescribed, based upon these values. However, in the recent past substantial between-laboratory variability has been present. This study evaluates the agreement of 25(OH)D measurement in four U.S. clinical laboratories and investigates the utility of a single calibrator as an approach to improve between-laboratory agreement.

METHODS

DEQAS 25-hydroxyvitamin D (25(OH)D) Data

- DEQAS = Vitamin D External Quality Assessment Scheme; a worldwide voluntary effort with over 200 laboratories participating
  - -Overall aim is to ensure analytical reliability of vitamin D assays
  - - Achieved through distribution of serum pools (n = 5) quarterly
- Various methodologies utilized by participating laboratories including Diasorin® (Stillwater, MN) radioimmunoassay; (RIA), high pressure liquid chromatography (HPLC) and liquid chromatography tandem mass spectroscopy (LCMSMS)
- Mean values reported; individual laboratory values available for HPLC and LCMSMS methods
- Data presented here from the April, 2006 distribution

Clinical 25(OH)D Measurement

- Serum obtained from 15 generally healthy adults (8 female, 7 male; age 20-60)
- A serum "calibrator" specimen known to contain ~30 ng/ml of 25(OH)D was utilized
- Aliquots sent as clinical specimens to four laboratories (Labs A-D) for 25(OH)D measurement
- Methodologies utilized for 25(OH)D measurement included HPLC (Lab A), LCMSMS (Labs B and C) and Diasorin RIA (Lab D)
- Inter-assay 25(OH)D measurement precision (%CV) obtained from each laboratory (Table 1)

Data Analysis

- Serum 25(OH)D results from laboratory A defined as the "gold standard" to which the others were compared based upon lowest %CV
- Vitamin D status defined by serum 25(OH)D measurement as follows:
  - - Deficiency; < 15 ng/ml
  - - Insufficiency; < 30 but ≥ 15 ng/ml
  - - Optimal; ≥ 30 ng/nl
- 25(OH)D measurements compared using linear regression and Bland-Altman analyses (Analyse-it, Leeds, UK)
- Systematic bias was assessed by determination of the difference of each measurement in labs B-D compared to lab A ("delta" 25(OH)D)
- For data "correction," each individual value from labs B-D was subsequently multiplied by the ratio of the "calibrator" value obtained in the respective lab compared to that obtained in lab A

RESULTS

DEQAS 25(OH)D Data

- Results arbitrarily presented for two sera (samples "1" and "2")
- For these two sera, there is good agreement of the mean 25(OH)D value from labs using Diasorin RIA (n = 54), HPLC (n = 9) and LCMSMS (n = 8) methodologies (Figure 1a)
- Between-laboratory variability is present for HPLC and LCMSMS when individual values are considered (Figure 1b)
- Evaluation by individual lab suggests that assay calibration differences may explain much of the between- laboratory difference (Figure 1c)

Clinical Analysis

- Diagnostic categorization (optimal, insufficient or deficient) was identical in 73% (11/15) of subjects (Figure 2)
- Good correlation was observed between lab A and the other three labs; R^2 = 0.99, 0.81 and 0.95 (Figures 3a-c)
  - - Modest between laboratory variability noted; mean bias ranged from +2.9 to +5.1 ng/ml (example in Figure 3d)
- This systematic positive bias leads to 89% (40/45) of values being higher in labs B-D than the corresponding lab A value (Figure 4)
- "Correction" of the values obtained by labs B-D reduces the mean bias in labs B and D (Figures 5a-c)
  - - After correction labs A and B obtain virtually identical results (Figures 5a and 5d)

FIGURES

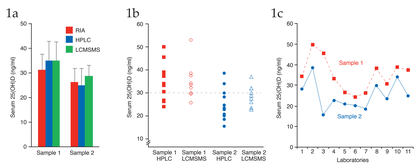

Figure 1a-c: ***DEQAS Data:*** There is good agreement of mean 25(OH)D concentration (1a) for these two serum specimens among the 71 reporting laboratories using the Diasorin RIA, HPLC or LCMSMS methods. 25(OH)D mean (SD) values (ng/ml) are as follows: Sample 1: 31.4 (6.4), 35.7 (8.4), 34.6 (7.3); Sample 2: 26.4 (5.6), 25.5 (7.1), 28.9 (5.0) for the RIA, HPLC and LCMSMS methods respectively. Data as mean (SD). However, when values are considered on an individual basis, substantial between laboratory variability is evident (1b). The dotted line depicts 30 ng/ml, a widely accepted definition of optimal vitamin D status. Assessment of individual values from the 11 reporting labs using HPLC suggests a systematic offset (1c).

Table 1: 25(OH)D Inter-Assay Precision (%CV)

25(OH)D Concentration	"Low"		"Mid"		"High"	
Laboratory	$25(OH)D_2$	$25(OH)D_3$	$25(OH)D_2$	$25(OH)D_3$	$25(OH)D_2$	$25(OH)D_3$
A	12.6	3.9	4.9	2.6	3.2	3.3
B	11.4	11.6	8.8	8.3	8.2	7.9
C	9.7	13.5	11.6	10.7	8.8	8.4
D	5.7		7.5			

FIGURES

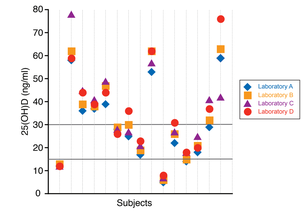

Figure 2: ***Vitamin D Diagnostic Categorization.*** The clinical assessment of vitamin D status (optimal, insufficient or deficient) was identical for 73% of these subjects in all four labs. However, one individual (7%) would be classified as deficient (in only one lab) and three individuals (20%) would be variably classified as optimal or insufficient.

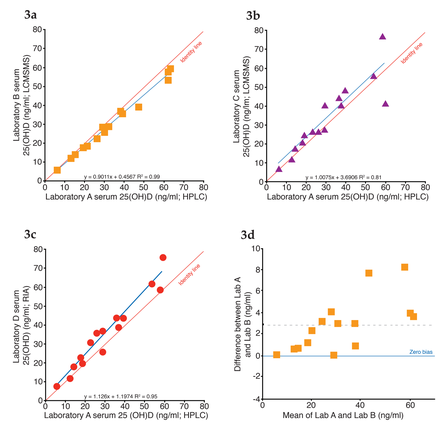

Figure 3a-d: ***Clinical 25(OH)D Results.*** Excellent correlation is observed between labs (Figure 3a-c). However, modest systematic bias is observed. Specifically, the mean bias compared with lab A is 2.9, 3.9 and 5.1 ng/ml for labs B, C and D respectively. This bias is clearly demonstrated by the Bland-Altman plot comparing labs A and B (Figure 3d).

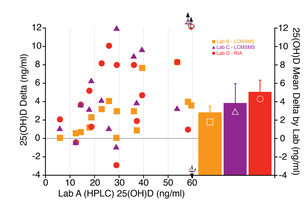

Figure 4: ***Demonstration of Systematic Bias.*** Support for between laboratory differences in assay calibration is gained by the demonstration that value of the vast majority (89%) of specimens from labs B, C and D are greater than the value from lab A. Data presented as "delta" 25(OH)D (the value obtained in lab B-D minus that obtained in lab A) versus the value obtained in lab A. Mean "delta" values (SEM) for labs B-D on right of figure.

FIGURES

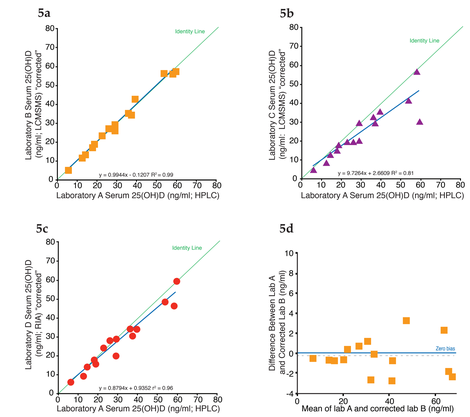

Figure 5a-c: ***Clinical 25(OH)D Results Utilizing a Correction Factor.*** Use of a single calibrator, in general, improves, but does not entirely resolve the between laboratory bias (Figures 5a-c). Specifically, the mean bias following "correction" of the values in comparison with lab A is -0.3, -5.7 and -2.8 ng/ml for labs B, C and D respectively. The correction produces nearly identical values for labs A and B (Figure 5d).

CONCLUSIONS

- Overall, there is good agreement between laboratories using HPLC, LCMSMS and RIA methodology to determine 25(OH)D, however modest variability persists.
- Based upon this variability, use of a "cutpoint"diagnostic approach (e.g., 30 ng/ml) will continue to variably identify individuals as having vitamin D inadequacy depending on the laboratory utilized.
- Systematic bias is present between laboratories, suggesting that use of calibrator(s) will enhance between laboratory agreement.
- Further measures to improve between clinical laboratory agreement appear likely to require measures to enhance 25(OH)D assay precision.