

Background

1,25-dihydroxyvitamin D [125D] is the active form of vitamin D and is produced primarily in the kidney by the hydroxylation of 25-hydroxyvitamin D (25D). Levels of 125D are increased in calcium-deficient individuals, children and during pregnancy and lactation. Compared to 25D, 125D circulates in the human serum at concentrations 1,000 folds lower, making its serum levels challenging to assess. We report the validation results of the automated IDS-iSYS 1,25-Dihydroxy vitamin D assay.

Methods

The accuracy profile was determined with 8 serum pool levels (9.3–175 pg/mL). Method comparison was performed using multiple kit lots; 260 serum samples were measured by IDS RIA and IDS-iSYS and 65 samples by IDS EIA and IDS-iSYS. In addition to establishing the reference intervals with specimens from apparently healthy subjects, we defined the observation ranges for the pediatric (stratified by every 2 years), pregnant women and hemodialysis patients.

Results

Accuracy profile

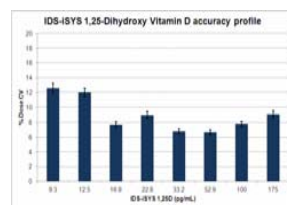


Fig. 1/ IDS-iSYS 1,25D Accuracy Profile.

Four (4) replicates of eight (8) serum pool levels (9.3 – 175 pg/mL) were extracted individually, then concentrated with a vacuum evaporator and measured each day, for 5 days (n=20). Excellent total precision was obtained, 7.7 – 12.6% CV; the error bar represented 1 SD.

Method comparison

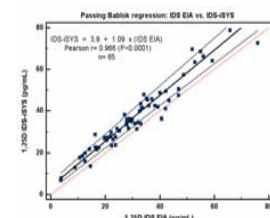


Fig. 2a. Passing Bablok regression: IDS EIA vs. IDS-iSYS.

65 serum samples were extracted per each method procedure. The regression slope (95% CI.) was 1.09 (1.01– 1.15) with correlation coefficient (r) (95%CI.) of 0.97 (0.94– 0.98).

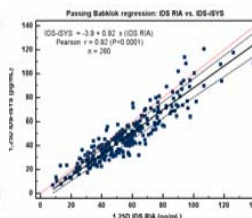


Fig. 2b. Passing Bablok regression: IDS RIA vs. IDS-iSYS.

Multiple kit lots were used to measure 260 serum samples. The samples were extracted per each method procedure. The regression slope (95% CI.) was 0.92 (0.87– 0.97) with correlation coefficient (r) (95%CI.) of 0.92 (0.90 – 0.94).

Apparent healthy adults reference intervals

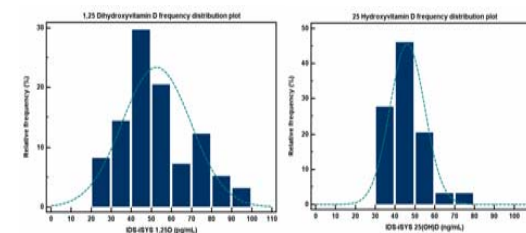


Fig. 3: Distribution of 1,25D and 25D in the apparent healthy, vitamin D sufficient study population (n=98).

The median 25D was 44.6 ng/mL (5-95 percentile 35.2-61.2), and median 1,25D was 49.2 pg/mL (5-95 percentile: 27.3-84.3). The IDS-iSYS 1,25D 95% reference interval calculated by a non-parametric percentile method (CLSI C28-A3) was 23.0-93.0 pg/mL.

Include vitamin D sufficiency as an inclusion criterion for healthy subjects, our Belgian population (n=98) had similar levels compared with the range of 26.1 – 95.0 pg/mL in healthy subjects provided by the manufacturer.

Pediatrics, pregnant women and hemodialysis ranges

Population	N	Mean (SD) (pg/mL)	Observed Range (pg/mL)
0-2 years	40	78.2 (33.0)	25.1 - 154
>2-4 years	40	61.2 (27.0)	21.8 - 156
>4-6 years	40	50.1 (20.4)	17.6 - 109
>6-8 years	40	46.1 (19.0)	17.8 - 80.0
>8-10 years	40	53.4 (17.2)	15.4 - 89.3
>10-12 years	40	40.5 (15.3)	15.1 - 72.4
>12-14 years	40	56.5 (20.1)	17.4 - 93.5
>14-16 years	40	55.9 (17.0)	31.5 - 88.2
>16-18 years	40	54.8 (14.2)	28.0 - 81.8
First trimester	42	60.4 (23.4)	18.7 – 124
Third trimester	42	142 (35.2)	40.4 - 207
Haemodialysis*	74	11.8 (6.8)	< 24.1

Table 1: Observed ranges of 1,25D circulating levels in pediatric, pregnant women and hemodialysis patients.

The 1,25D concentration levels are higher in children than adults. 3rd trimester pregnant women have the highest 1,25D circulating levels. As anticipated, the hemodialysis patients poorly hydroxylate 25D to 1,25D, leading to low 1,25D levels.

Conclusion

- Our data indicate that the automated IDS-iSYS 1,25-Dihydroxy Vitamin D method offers a reliable alternative for laboratories currently using the manual immunoassay.
- With excellent accuracy profile and good correlation to the existing manual immunoassays, the IDS-iSYS 1,25D is an accurate method for the measurement of 1,25-dihydroxyvitamin D concentration.
- With 25(OH) Vitamin D sufficiency as inclusion criteria in addition to normal calcium, phosphates and PTH, we obtain similar reference intervals for apparent healthy Belgium subjects compared with the manufacturer range.
- The established 1,25-dihydroxy vitamin D observation ranges for different populations will be a valuable tool for clinical laboratories and clinicians to assess the 1,25D status.