Development of an automated assay for the measurement of 1,25 dihydroxyvitamin D on the LIAISON® analyzer

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Introduction

1,25 dihydroxyvitamin D (1,25-$(OH)_2$-D) is the most potent naturally occurring Vitamin D metabolite in the body therefore its production in the kidney is tightly regulated through serum concentrations of calcium, phosphorus, and parathyroid hormone. In addition to this well characterized relationship with parathyroid hormone and the regulation of bone mineral metabolism, recent evidence also indicates 1,25-$(OH)_2$-D has important roles in cancer, inflammation, and immunity[1,2,3,4,5]. Altered serum levels of 1,25-$(OH)_2$-D in various disease states such as Vitamin D deficiency and intoxication, hyper and hypoparathyroidism, renal failure, granulomatous diseases, and malignancies demonstrate the increasing importance of researching these mechanisms. Consequently the measurement of 1,25-$(OH)_2$-D is rapidly becoming an important tool in the research and management of many diseases and conditions besides those that affect the normal metabolism of phosphorus and calcium.

We report here the development of an automated non-isotopic immunoassay for 1,25 dihydroxyvitamin D on the LIAISON® analyzer.

Material methods

LIAISON® 1,25 Dihydroxyvitamin D Kit (DiaSorin Inc., Stillwater, MN 55082)
LIAISON® Analyzer

The DiaSorin 1,25-$(OH)_2$-D assay consists of a two-step procedure. The assay involves preliminary extraction and subsequent purification of Vitamin D metabolites from 500 µL serum or EDTA plasma using C18OH "Extra Clean" cartridges. Following the extraction the treated sample is then assayed using a competitive chemiluminescent immunoassay on the LIAISON® automated analyzer. Once on board 200 µL of treated sample is loaded from the sample area into a LIAISON® reaction module where 1,25-$(OH)_2$-D is bound by anti-1,25 Dihydroxyvitamin D antibody coated paramagnetic particles. After 30 minutes incubation at 37°C the 1,25-$(OH)_2$-D isoluminal derivative linked conjugate is added. After a second, 10minute incubation, unbound reagent is removed via a wash cycle. The reaction module is then transported into the counting chamber which automatically injects trigger to initiate the chemiluminescent reaction. Measurement for the LIAISON® 1,25 Dihydroxyvitamin D assay is then reported between 5 and 200 pg/mL. Total time to first result is approximately 55 minutes with a throughput of 90 samples per hour.

Amount	Component
200 µL	Extracted calibrators, controls, or samples
+ 20 µL	Coated magnetic particles
30 min	Incubation
+ 45 µL	Conjugate
10 min	Incubation followed by wash cycle
3 s	Measurement

Results and discussion

Performance characteristics of the assay were determined in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI) from standard CLSI protocols.

Sensitivity

When defined as the concentration 2 standard deviations from the RLU at maximum binding, the minimum detectable amount is ≤ 4 pg/mL.

Precision

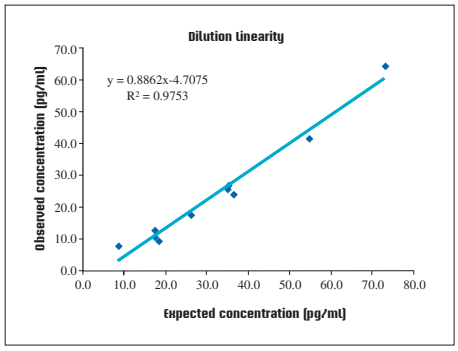
Four samples, containing different concentrations of 1,25-$(OH)_2$-D, were assayed in quadruplicate, 1 run per day over 5 operating days, to determine the repeatability and reproducibility of the assay (i.e. within- and between-assay variability).

Intra-Assay Precision	1	2	3	4
Number of determinations	20	20	20	20
Mean (pg/mL)	20.7	44.0	85.5	34.2
Intra SD (pg/mL)	2.3	4.5	9.4	3.5
Coefficient of variation (%)	11%	10%	11%	10%

Inter-Assay Precision	1	2	3	4
Number of determinations	20	20	20	20
Mean (pg/mL)	20.7	44.0	85.5	34.2
Grand SD (pg/mL)	2.9	4.7	9.5	3.6
Coefficient of variation (%)	14%	11%	11%	10%

Dilution Linearity

Three patient samples were diluted with the zero calibrator and analyzed. Percent Expected vs. Observed was calculated from mean values of neat and diluted samples. A regression of Expected vs. Observed values is shown below.



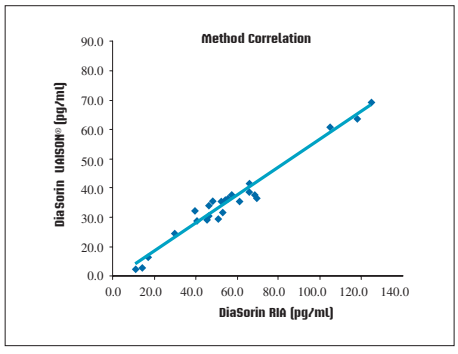
Recovery

Three high concentration serum samples and three low concentration serum samples were blended together at three ratios. Neat serums, along with the blends, were analyzed in a single assay. The observed values were compared to the expected values to determine the % recovery. The results are calculated using the equation: % recovery = observed/expected.

	Defined	Expected	Observed	% Recovery
High Sample 1 (HS1)	60.3			
2 HS1 : 1 LS1		55.5	48.1	87%
1 HS1 : 1 LS1		53.0	59.2	112%
1 HS1 : 2 LS1		50.6	56.1	111%
Low Sample 1 (LS1)	45.8			
High Sample 2 (HS2)	78.5			
2 HS2 : 1 LS2		63.5	59.9	94%
1 HS2 : 1 LS2		55.7	57.4	103%
1 HS2 : 2 LS2		48.0	47.3	99%
Low Sample 2 (LS2)	32.9			
High Sample 3 (HS3)	138.9			
2 HS3 : 1 LS3		99.8	104.3	105%
1 HS3 : 1 LS3		79.6	91.6	115%
1 HS3 : 2 LS3		59.4	60.8	102%
Low Sample 3 (LS3)	20.3			
			Mean Recovery	103%
			SD	0.090

Method Comparison

1,25-$(OH)_2$-D levels in a total of 25 clinical samples were tested by the LIAISON® 1,25 Dihydroxyvitamin D Assay and by DiaSorin 1,25-Dihydroxyvitamin D ^{125}I RIA.
The linear regression equation obtained was: LIAISON® = 0.954 (RIA) – 3.00; R = 0.98.



Conclusions

These results demonstrate that the LIAISON® 1,25-Dihydroxyvitamin D assay measures 1,25-$(OH)_2$-D with good accuracy and precision . In addition the correlation to the DiaSorin 1,25-$(OH)_2$-D RIA demonstrated a very close association between the two test methods. With these performance characteristics and a time to first result of one hour and throughput of 90 tests per hour, the DiaSorin LIAISON® 1,25 Dihydroxyvitamin D assay offers a convenient method for the routine measurement of 1,25 dihydroxyvitamin D in an automated, non-isotopic immunoassay.

References:

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