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Development of a Vitamin D Total Assay* with LOCI Technology on the Dimension EXL Integrated Chemistry System

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Introduction

The Siemens Dimension® EXL™ Integrated Chemistry System incorporates multiple detection technologies, including LOCI® technology, which enables high-sensitivity immunoassay formats. Siemens is currently developing a vitamin D total assay utilizing LOCI technology on the Dimension EXL system.

The Dimension EXL LOCI Vitamin D Total assay* is a homogeneous, chemiluminescent immunoassay. The LOCI reagents include a releasing reagent, two synthetic bead reagents, and a biotinylated monoclonal antibody reagent.

The assay requires 8 µL of serum or plasma and is linear from 4 to 150 ng/mL. Time to first result is 28.7 minutes, with calibration stable for 7 days. Repeatability and within-lab CVs were less than or equal to 2.9% and 5.1% respectively between 10 and 100 ng/mL.

Immunometric Format

The first bead reagent (Sensibeads) is coated with streptavidin and contains photosensitive dye. The second bead reagent (Chemibeads) is coated with a 25(OH)vitamin D_3 analog and contains chemiluminescent dye. Sample is incubated with the releasing reagent to release the 25(OH)vitamin D molecules from the vitamin D-binding proteins. The reaction mixture containing released 25(OH)vitamin D molecules is then incubated with biotinylated antibody to form a 25(OH)vitamin D/biotinylated antibody complex. Chemibeads coated with the 25(OH) vitamin D_3 analog are added to scavenge the excess free biotinylated antibody. Streptavidin-coated Sensibeads are then added and bind to the biotin portion of the biotinylated antibody. Aggregates of Chemibead analog/biotinylated antibody/streptavidin-coated Sensibeads are formed as a result. Illumination of the reaction mixture by light at 680 nm generates singlet oxygen from the Sensibeads, which diffuses into the Chemibeads and triggers a chemiluminescent reaction. The resulting chemiluminescent signal is measured at 612 nm and is inversely proportional to the concentration of 25(OH)vitamin D in the sample.

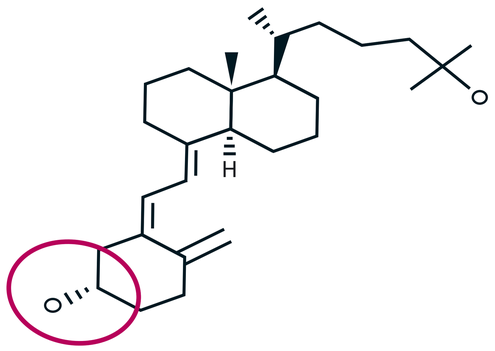

Figure 1. A 25(OH)vitamin D_3 analog is conjugated to the Chemibeads.

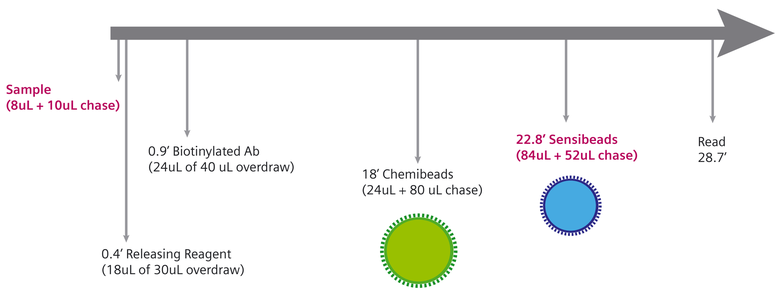

Figure 2. Reaction scheme of the LOCI Vitamin D Total assay.

Precision

Samples tested include UTAK controls and human sera spiked with 25(OH)vitamin D_3 or without spike at concentrations shown in Table 1.

Table 1. Precision at different levels.

Attribute	Current Performance
Repeatability	2.2%@ ~15 ng/mL (Serum #3)
	1.9% @ ~18 ng/mL (UTAK Low QC)
	1.6% @ ~27 ng/mL (Serum #23)
	1.5% @ ~34 ng/mL (Serum #4)
	1.7% @ ~41 ng/mL (UTAK Plus 1 QC)
	2.0% @ ~53 ng/mL (Serum #29)
	2.5% @ ~88 ng/mL (UTAK Plus 2 QC)
	2.9% @ ~95 ng/mL (80% spike)
Within Laboratory Precision	3.1%@ ~15 ng/mL (Serum #3)
	2.8% @ ~18 ng/mL (UTAK Low QC)
	2.3% @ ~27 ng/mL (Serum #23)
	2.3% @ ~34 ng/mL (Serum #4)
	2.4% @ ~41 ng/mL (UTAK Plus 1 QC)
	2.8% @ ~53 ng/mL (Serum #29)
	3.2% @ ~88 ng/mL (UTAK Plus 2 QC)
	5.1% @ ~95 ng/mL (80% spike)

25(OH)vitamin D_2/D_3 Equimolarity

30 µg/mL of 25(OH)vitamin D_2 and D_3 ware spiked individually into two serum samples with baseline values about 25–30 and 50–60 ng/mL.

Table 2. 25(OH)vitamin D_2/D_3 equimolarity measured at two levels.

Compound	Spiking conc. (ng/mL)	Serum sample (25-30 ng/mL)	Equimolar	High level 25(OH) vitamin D serum (50-60 ng/mL)	Equimolar
25(OH) Vitamin D_2	30	53.3	102%	82.8	100%
25(OH) Vitamin D_3	30	50.9		80.5	

Method Comparison 1: Dimension EXL Assay vs. Ghent University ID-LC/MS/MS

The Dimension EXL Vitamin D Total assay was compared to the ID-LC/MS/MS 25(OH)vitamin D reference measurement procedure (RMP) from the University of Ghent. Ninety patients' sera ranging from 5.8 to 79.2 ng/mL were tested in this study.

Forty samples were from the CDC's Vitamin D Standardization-Certification Program (VDSCP), which already contained original values from the University of Ghent. Fifty samples were sent for measurement at the University of Ghent.

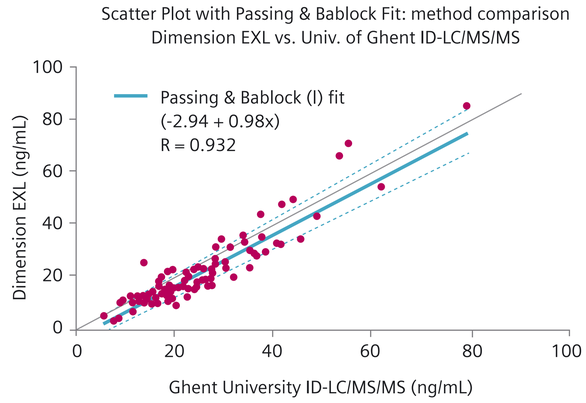

Figure 3. Method comparison: Dimension EXL assay vs. Univ. of Ghent ID-LC/MS/MS

Method Comparison 2: EXL vs. ADVIA Centaur Vitamin D Assay

The study employed 346 patients' sera, including 32 vitamin D_2-supplemented patients and 17 kidney-failure patients under dialysis medical care.

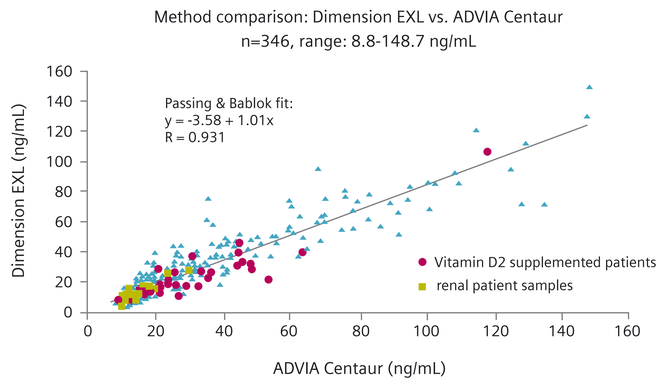

Figure 4. Method comparison: Dimension EXL vs. ADVIA Centaur.

Dilutional Linearity

Human serum containing 152 ng/mL of 25(OH) vitamin D was diluted with multidiluent. The diluted samples were tested in duplicate. Linear regression of the mean observed results (Y) versus the theoretical result (X) showed good recovery across the assay range.

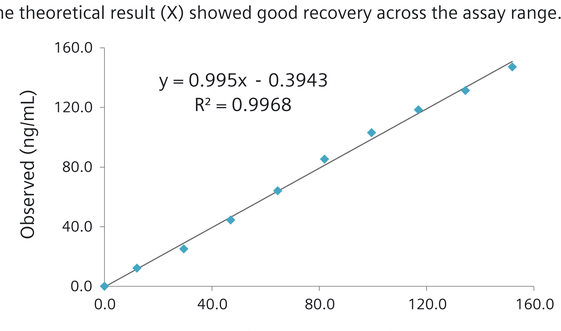

Figure 5. Linearity at nine different levels.

3-Epi-25(OH)vitamin D_3 Cross-reactivity

The Dimension EXL Vitamin D Total assay incorporates a proprietary 3-epimer blocker antibody that lowered the average cross-reactivity from 14% to <2%.

Table 3. Effect of 3-epimer blocker Ab on cross-reactivity.

Sample ID	No 3-epimer blocker Ab		With 100ug/mL of 3-epimer block Ab	
	Serum value (ng/mL)	Cross-Reactivity (%)	Serum value (ng/mL)	Cross-Reactivity (%)
S2 S2-spiked 3epi	30.2 48.0	17.9%	30.7 33.0	2.2%
S3 S3-spiked 3epi	22.5 40.5	17.9%	23.2 25.6	2.4%
S5 S5-spiked 3epi	19.8 31.6	11.9%	20.7 22.5	1.8%
S6 S6-spiked 3epi	40.4 53.0	12.6%	40.5 41.4	1.0%
S7 S7-spiked 3epi	46.6 62.7	16.1%	45.6 49.2	3.5%
S9 S9-spiked 3epi	47.3 57.5	10.2%	48.5 46.4	-2.0%
S10 S10-spiked 3epi	53.1 62.2	9.1%	55.4 55.4	0.0%
S11 S11-spiked 3epi	39.5 50.0	10.5%	40.8 43.8	3.0%
S33 S33-spiked 3epi	47.4 62.8	15.3%	48.6 50.8	2.2%
S41 S41-spiked 3epi	23.6 39.1	15.5%	24.8 28.6	3.8%
Avg. Cross-reactivity%		**13.7%**		**1.8%**

Lot to Lot comparison: Lot A (with the 3-epimer blocker Ab) vs. Lot B (without the 3-epimer blocker Ab). Both lots showed that anti-3-epimer mAb neither changed the curve shape nor affected the patient recovery, indicating low cross-reactivity to 25(OH)vitamin D. 353 patients' sera ranging from 3.8 to 149.1 ng/mL were tested in this study.

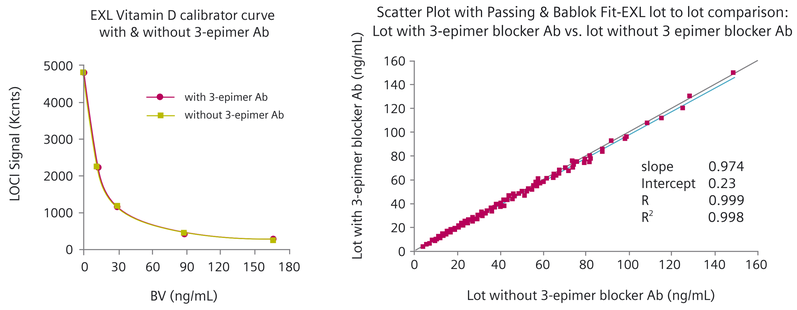

Figure 6. Comparison of calibration curve and sample recovery with and without 3-epimer blocker Ab.

Conclusions

The Dimension EXL Vitamin D Total assay demonstrates acceptable precision, accuracy, and minimal cross-reactivity to the 3-epimer for total 25(OH)vitamin D measurement on the Dimension EXL system.

*Not available for sale. Due to local regulations, not all products will become available in all countries.