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

J Li, E Garcia, Z Teng, M Drinan, R Janzen, C Larson, M Stranz, D Clark, B Wessel, P Singh, M Sharma, TQ Wie, R Molinaro
Siemens Healthcare Diagnostics Inc. Newark, Delaware

Introduction
The Siemens Dimension® EXL™ Integrated Chemistry System incorporates multiple detection technologies, including LOCI® technology, which enables high-sensitivity immunoassay format. 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.

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 D₃ 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 D₃ 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 chemiluminescence. The resulting chemiluminescent signal is measured at 612 nm.

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.

Conclusions
The Dimension EXL LOCI Vitamin D Total assay demonstrates acceptable precision, accuracy, and minimal cross-reactivity to the 3-epimer of 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.

Table 1. Precision at different levels.

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Current Performance</th>
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<tbody>
<tr>
<td>Repeatability</td>
<td></td>
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<tr>
<td>Within Laboratory Precision</td>
<td></td>
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25(OH)vitamin D₃/D₇ Equimolarity
30 µg/mL of 25(OH) vitamin D₃ and D₇ were spiked individually into two serum samples with baseline values about 25–30 and 50–60 ng/mL.

Table 2. 25(OH) vitamin D₃/D₇ equimolarity measured at two levels.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Spiking conc. (ng/mL)</th>
<th>Serum samples (25-30 ng/mL)</th>
<th>Equimolar</th>
<th>High level (25(OH) vitamin D₇) serum (50-60 ng/mL)</th>
<th>Equimolar</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH) vitamin D₇</td>
<td>100</td>
<td>100</td>
<td>100%</td>
<td>82.0</td>
<td>100%</td>
</tr>
<tr>
<td>25(OH) vitamin D₃</td>
<td>50</td>
<td>50</td>
<td>93.5</td>
<td>83.5</td>
<td>83.5</td>
</tr>
</tbody>
</table>

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 D₃ or without spike at concentrations shown in Table 1.

Figure 1. A 25(OH) vitamin D₃ analog is conjugated to the Chemibeads.

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

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

Figure 5. Linearity at nine different levels.

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

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