Enzyme immunoassay for measuring 25-hydroxyvitamin D₃ in serum

Charlotte Lind, Jiewei Chen, and Inger Byrjalsen*

We developed a rapid, competitive enzyme immunoassay (EIA) for measuring 25-hydroxyvitamin D₃ [25(OH)D₃] in serum. The EIA was based upon 25(OH)D₃-3-hemisuccinate covalently coupled to secondary amino groups grafted onto the polystyrene surface of microtiter wells. Optimal coupling conditions were established, and we found that inclusion of 40 μmol/L chloramine T, an agent not previously described for use in coupling to these plates, resulted in both more reproducible coupling as well as more than a twofold increase in the coupling efficiency. Before EIA, 25(OH)D₃ was extracted from the serum samples by acetonitrile, and the redissolved extract was incubated with polyclonal rabbit antibody raised against 1,25-dihydroxyvitamin D₃-3-hemisuccinate conjugated to bovine serum albumin. Peroxidase-labeled antibody raised in goat against rabbit immunoglobulins was used for detection. The detection limit of the EIA was 4.4 μg/L; recovery 102%; on-plate CV 11%; within-run CV including extraction 12%, and between-run CV 15%. There was no clinically important cross-reactivity with other vitamin D metabolites, and results obtained by the EIA were compared with results obtained by a previously described RIA.

INDEXING TERMS: vitamin D status • steroid hormones • cholecalciferol • EIA • covalent coupling

Vitamin D in its physiologically active form is a secosteroid hormone that is involved in the active intestinal absorption of calcium and in the regulation of calcium homeostasis [1, 2]. Vitamin D exists in two main types, vitamin D₂ and vitamin D₃, which have slightly different side chains. Vitamin D₂, mainly a synthetic product, is almost exclusively obtained via fortified dairy products and hence the contribution of this metabolite in the diet is very low. Vitamin D₃ is supplied via the diet or is produced in the skin by exposure to ultraviolet light [3, 4]. In the liver, vitamin D is hydroxylated to produce 25-hydroxyvitamin D [25(OH)D] [2], the predominant circulating form of vitamin D. The concentration in serum of this metabolite reflects the general status of the organism with regard to the availability of vitamin D [5]. Although the biologically active metabolite is 1,25-dihydroxyvitamin D [1,25(OH)₂D], the concentration of circulating 25(OH)D provides better information with respect to the patient’s vitamin D status than that of 1,25(OH)₂D [5]. The concentration in serum of 25(OH)D decreases with age [6], and deficiency is common among the elderly [3, 7]. This is due both to reduced sunlight exposure, decreased production in the aging skin [8], and inadequate nutritional vitamin D intake. Vitamin D deficiency is believed to increase the risk of osteoporosis, a systemic skeletal disease characterized by low bone mass and increased susceptibility to fracture.

Several analytical principles have been applied for the measurement of 25(OH)D in serum or plasma, such as HPLC [9–11], competitive protein-binding assay [11–16], and RIA [17–19]. The HPLC methods are very time consuming, requiring extensive purification steps, and the competitive protein-binding assays require both solvent extraction and chromatographic purification before assay. The introduction of antibody preparations against vitamin D metabolites made it possible to develop assays that generally are more specific vitamin D assays, thus requiring fewer purification steps. Traditionally, the RIAs have been based on a ³H-labeled tracer, but the use of a ¹²⁵I-labeled tracer has been described, thus avoiding the time-consuming liquid scintillation counting [19].

An enzyme immunoassay (EIA) would represent a new approach to perform vitamin D analysis. Microtiter
plates with secondary amino groups grafted onto the polystyrene surface have been introduced, giving the opportunity of covalent coupling of peptides or steroid derivatives to the surface [20, 21]. We report here the development and assay performance of the first described competitive EIA for the measurement of 25(OH)D₃ in serum with this new approach of covalent linkage of a hemisuccinate derivative of 25(OH)D₃ to the microtiter plates.

Materials and Methods

Materials
CovaLink™ Modules and PolySorp microtiter plates (mixing plates) were purchased from Nunc (Roskilde, Denmark). Crystalline 25(OH)D₃ used for derivatization was kindly donated by M.R. Uskokovic, Hoffmann-La Roche (Nutley, NJ). A 3-hemisuccinate derivative of the 25(OH)D₃ was synthesized by K J Ross-Petersen (Hørsholm, Denmark) according to the procedure of Lichtwald et al. [22]. A previously described antisera raised in rabbits against 1,25(OH)₂D₃-3-hemisuccinate conjugated to bovine serum albumin (BSA) was used [17]. Goat anti-rabbit peroxidase-conjugated immunoglobulin was purchased from Jackson ImmunoResearch Labs. (West Grove, PA), and tetramethyl benzidine (TMB) peroxidase substrate system was purchased from Kirkegaard & Perry Labs. (Gaithersburg, MD). Skim milk powder was acquired from Difco Labs. (Detroit, MI) and horse serum [intrinsically low in 25(OH)D] enriched with 25(OH)D₃ was used as calibrator. The calibrator was prepared (twofold serial dilutions, 175 to 2.7 µg/L) each day of analysis. Extraction of 25(OH)D₃ from the serum samples and the calibrators was performed by slowly pipetting 100 µL of sample below the surface of 500 µL of acetonitrile in conical glass tubes. The tubes were vortex-mixed for 15 s, left for 15 min, and centrifuged at 3000g for 5 min at 4 °C. Four hundred microliters of the supernatant was transferred to conical glass tubes, evaporated to dryness under air at 40 °C, and redissolved in 20 µL of acetonitrile. The redissolved extract was further diluted with 180 µL of assay buffer before assay.

EIA procedure

Incubations and preincubations were done in wells of microtiter plates, covered with sealing tape on a horizontal rotating shaker at 250 to 300 rpm (InterMed, Roskilde, Denmark). All determinations were performed in duplicate and all steps were carried out at room temperature. Seventy-five microliters of redissolved sample or calibrator extract was pipetted into the well of a mixing plate and incubated with 75 µL of 1,25(OH)₂D₃ antiserum diluted 1:1000 in assay buffer. The mixture was preincubated for 2 h and 100 µL of this incubation mixture was transferred to the prepared EIA plate and then incubated for 1 h. The wells were emptied and washed four times with washing buffer. Peroxidase-labeled antibody raised against rabbit immunoglobulins was diluted 1:500 in 0.1 mol/L NaH₂PO₄·H₂O, 50 g/L skim milk powder, 5 g/L BSA, pH 7.2.

Human serum samples were obtained from in-house employees ages 25 to 40 years and healthy volunteers ages 70 to 78 years, who gave their informed consent for the analysis.

Coupling of 25(OH)D₃ to the microtiter plate

Immediately before preactivation, 100 µL of freshly prepared 3.75 mol/L N-hydroxysuccinimide (NHS) was gently mixed with an equal volume of freshly prepared 7.5 mol/L 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC). For preactivation, 45 µL of 25(OH)D₃-3-hemisuccinate (10 µg/100 µL in 1,4-dioxane) was gently mixed with 90 µL of the NHS-EDC solution and left for 20 to 40 min at room temperature. The preactivated mixture was diluted with 11.25 mL of PBS containing 40 µmol/L chloramine T, and 100 µL was applied per well of the 96-well CovaLink plate. The plate was covered with sealing tape and left for covalent coupling overnight at room temperature. After coupling, the wells were emptied and short-time blocked with 200 µL of PBS containing 50 g/L skim milk powder and 10 µL/L Tween 20 for 2 to 4 min, without shaking. The wells were emptied and further blocked by addition of 200 µL of PBS containing 50 g/L skim milk powder. The plate was blocked for 1 h and the EIA plate was used the same day or stored in a foil bag with a drying capsule at 4 °C.

Sample extraction procedure

Horse serum [intrinsically low in 25(OH)D] enriched with 25(OH)D₃ was used as calibrator. The calibrator was prepared (twofold serial dilutions, 175 to 2.7 µg/L) each day of analysis. Extraction of 25(OH)D₃ from the serum samples and the calibrators was performed by slowly pipetting 100 µL of sample below the surface of 500 µL of acetonitrile in conical glass tubes. The tubes were vortex-mixed for 15 s, left for 15 min, and centrifuged at 3000g for 5 min at 4 °C. Four hundred microliters of the supernatant was transferred to conical glass tubes, evaporated to dryness under air at 40 °C, and redissolved in 20 µL of acetonitrile. The redissolved extract was further diluted with 180 µL of assay buffer before assay.

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Cross-reactivity

For estimation of cross-reactivity, different vitamin D metabolites were dissolved in absolute ethanol and spectrophotometrically quantified at 265 nm. Each metabolite was further diluted in 500 mL/L ethanol to produce the desired concentration range. Aliquots of these solutions were dried under air and subsequently redissolved as previously described in the sample extraction procedure. The calibration curve of each metabolite was drawn and
the concentration that equalled the signal corresponding to 50% displacement of the 25(OH)D₃ was read. The cross-reactivity was defined as the ratio between the read concentration of 25(OH)D₃ and the test metabolite.

LIPID INTERFERENCE
For estimation of lipid interference, a pool of hyperlipidemic rabbit sera was mixed in six ratios with ultracentrifuged sera of the same pool, thereby producing identical serum samples but with increasing degree of lipemia. A human serum sample was enriched with these six mixtures of rabbit sera (75% human serum and 25% rabbit serum). The rabbit mixtures and the enriched human serum sample were measured in the EIA, and total cholesterol and triglycerides were determined by standard routine procedures. This procedure was carried out twice with different rabbit and human serum samples.

STATISTICAL ANALYSIS
SAS Institute procedures were used for statistical analysis [23]. The EIA results were compared with results obtained by RIA, in which serum samples were extracted with acetonitrile and subsequently chromatographically purified as described elsewhere [17]. The significance of the mean differences between groups was assessed by Student’s t-test for paired data and regression lines were based on orthogonal regression (Deming’s linear regression) [24].

Results
COUPLING OF 25(OH)D₃ TO THE MICROTITER PLATE
Optimal coupling conditions were determined by initial experiments in which maximum signal, i.e., the signal of the zero calibrator (assay buffer), was compared with the remaining nonspecific signal after displacement with 360 μg/L 25(OH)D₃ diluted directly in assay buffer.

The coupling efficiency was dependent on the concentrations of EDC and NHS and preactivation. Optimal preactivation conditions were determined by combining serial dilutions of EDC with serial dilutions of NHS, and the optimal concentrations in the preactivation mixture were found to be 2.5 mol/L EDC and 1.25 mol/L NHS. These concentrations were lowered by dilution in PBS before transferring to the microtiter wells. Initial experiments without preactivation showed very low and insufficient coupling efficiency. Generally, the coupling efficiency was lowered if the EDC and NHS were not prepared immediately before use. The addition of chloramine T to the PBS made the coupling procedure more reproducible and also increased the coupling efficiency. As seen in Fig. 1, more than a twofold increase in maximum signal was observed when including 20 to 80 μmol/L chloramine T in the coupling mixture, whereas no increase in nonspecific signal was observed. The effect of adding from 0 to 80 ng/100 μL of 25(OH)D₃-3-hemisuccinate to the microtiter well is shown in Fig. 2. Increasing coupling concentration increased the maximum signal until it leveled off. Similar maximum coupling was obtained with 40 to 80 ng per 100 μL, and coupling with 40 ng/100 μL equal to $7.9 \times 10^{-11}$ mol/100 μL was selected. When all these molecules were coupled, 48% of the functional groups of the well were occupied, as each well contains $1.7 \times 10^{-10}$ mol functional groups [21].

After coupling of the 25(OH)D₃-3-hemisuccinate, the
wells were blocked with PBS containing skim milk powder to decrease the nonspecific binding of the antiserum. Short-time blocking with 10 μL/L Tween 20 in PBS containing 50 g/L skim milk powder before blocking increased the maximum signal, while the nonspecific signal was not increased.

The prepared EIA plates could be stored at 4 °C in sealed foil bags with a drying capsule without loss of reactivity for at least 3 months.

**SAMPLE EXTRACTION PROCEDURE**

The effect of the serum volume on extraction recovery was examined to ensure sufficient and reproducible extraction recovery. This experiment was based on [3H]25(OH)D$_3$-labeled serum samples that were either extracted with acetonitrile or diluted with PBS. Volumes of serum ranging from 25 to 200 μL were extracted with the same efficiency of 83% ± 2.8% (mean ± SD). When 250 μL of serum was extracted, the extraction recovery decreased to 66%, so in the final version of the EIA we chose a volume of 100 μL of serum for extraction. Correction for extraction recovery was omitted because the extraction recovery was reproducible and calibrators were extracted in parallel to the samples in the assay.

The dried sample extract was redissolved in 20 μL of acetonitrile before further dilution with 180 μL of assay buffer. If ≥40 μL of acetonitrile was used for redissolution, the nonspecific signal of the EIA increased. Redissolving the dried extract in acetonitrile before adding the assay buffer was important to dissolve the 25(OH)D$_3$. If the acetonitrile and assay buffer were added simultaneously, less than half of the 25(OH)D$_3$ was redissolved, as compared with the sequential addition. Acetonitrile was chosen for redissolution, since ethanol increased the nonspecific signal of the EIA.

**ASSAY PERFORMANCE**

In an assay buffer system without skim milk powder and BSA, the nonspecific binding of the primary antibody was pronounced, whereas the addition of skim milk powder and BSA diminished this effect. On the other hand there was no detectable problem with nonspecific binding of the secondary antibody, so this antibody was incubated in assay buffer without skim milk powder and BSA. The effect on the signal of the incubation temperature was examined. Incubation of the antiserum at room temperature and 4 °C gave comparable results, and incubation at room temperature was chosen. To increase the sensitivity and thereby allow measurement of samples within the reference interval, sequential incubation, i.e., preincubation at a mixing plate before incubation at the EIA plate, was examined. When using sequential incubation, the detection limit decreased approximately five times compared with direct incubation. The detection limit became 4.4 ± 2.4 μg/L (mean ± SD, on the basis of three experiments), defined as the concentration corresponding to the mean absorbance of 10 determinations of the zero calibrator – 2SD of the absorbance. The calibration curve was approximately linear in the range 15–50 μg/L when plotted on a log-linear basis. Fig. 3 shows a typical calibration curve and serial dilutions of four samples. The serum samples were extracted and subsequently diluted in assay buffer before analysis. The curves of the diluted samples were parallel to the calibration curve, demonstrating that human 25(OH)D$_3$ (in serum samples) and 25(OH)D$_3$ (in the calibrator) had the same ability to displace the signal in the EIA. Similar results were obtained when the serum samples were diluted with horse serum before extraction.

Estimation of imprecision was performed by measurements of serum samples analyzed on different days. The result is given as the mean CV (Table 1). For data in the reference range (15–50 μg/L) the within-run CV on the plate was 11%, calculated from duplicate determinations of 139 samples measured in 10 days, 5 to 22 samples each day. The within-run CV including the extraction procedure was 12%, determined by analysis of 21 samples distributed over 9 days, extracted twice and subsequently measured in the EIA. The between-run CV was 15%, based on repeated measurements of three serum samples ranging 15 to 43 μg/L, which were extracted and analyzed in 6 to 10 runs over 4 1/2 months. The analytical

![Figure 3](image)

**Table 1. Imprecision of the 25(OH)D$_3$ EIA in the reference interval.**

<table>
<thead>
<tr>
<th></th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within-run</td>
<td>11</td>
</tr>
<tr>
<td>Within-run incl. extraction</td>
<td>12</td>
</tr>
<tr>
<td>Between-run</td>
<td>15</td>
</tr>
</tbody>
</table>
recovery of the EIA was determined by adding diminutive volumes of the 25(OH)D₃ calibration doses corresponding to 10, 12.5, 20, 25, 40, and 50 µg/L to four serum samples. These enriched samples were equilibrated for 30 min, stored at −20 °C, and extracted and assayed in the EIA in 3 to 6 different days. Estimation of analytical recovery was performed by dividing sample differences with the added concentrations. The overall analytical recovery of 25(OH)D₃ was 102% ± 13% (mean ± SD) (Table 2).

The specificity of this EIA was assessed by measuring the extent to which related vitamin D metabolites could displace the signal. The vitamin D metabolites were measured simultaneously in the EIA, and the calibration curves were drawn. The concentration of each metabolite that was required to reach 50% displacement, i.e., the mean of total and nonspecific signal, was compared with that of 25(OH)D₃. The result is given as the mean value of two experiments. Minimal cross-reactivity was seen with vitamin D₂ metabolites, whereas the cross-reactivity with vitamin D₃ metabolites was higher. 24,25(OH)₂D₃ competes half as much as 25(OH)D₃, and 1,25(OH)₂D₃, which in serum is 1000-fold lower in abundance than 25(OH)D₃, competes 10-fold better than 25(OH)D₃ (Table 3).

To examine to what extent the EIA was affected by lipid interference, identical serum samples but with increasing degree of lipemia were measured in the EIA. The concentration of 25(OH)D₃ was unaffected by increased degree of lipemia, i.e., the mean of total and nonspecific signal, was compared with that of 25(OH)D₃. The result is given as the mean value of two experiments. Minimal cross-reactivity was seen with vitamin D₂ metabolites, whereas the cross-reactivity with vitamin D₃ metabolites was higher. 24,25(OH)₂D₃ competes half as much as 25(OH)D₃, and 1,25(OH)₂D₃, which in serum is 1000-fold lower in abundance than 25(OH)D₃, competes 10-fold better than 25(OH)D₃ (Table 3).

The EIA was compared with a RIA in which serum samples were extracted with acetonitrile and subsequently chromatographically purified [17]. Both assays were based on the same antibody preparation. When values obtained in the EIA were plotted against values obtained in the RIA, a linear relation was observed. The slope of the orthogonal regression line was 1.27 ± 0.12 (mean ± SD) and the intercept was −2.3 ± 0.1 µg/L (r = 0.76) (Fig. 4). The mean serum concentration of 25(OH)D₃ was 35.2 µg/L when estimated in the EIA and 29.6 µg/L when estimated in the RIA. This rather small difference (5.6 µg/L) was, however, highly significantly different from zero by a t-test for paired data (P <0.001).

### Discussion

A traditional ELISA is based on passive adsorption of antibodies to a plastic surface. Immobilization of antigens on the microtiter well, a widely used support in solid-phase assays, permits the development of other types of EIA. Immobilization of small haptenic molecules and steroid molecules such as 25(OH)D₃, which bind only weakly, or not at all, by simple physical adsorption, involves coupling to a carrier or covalent coupling. The covalent binding obviates the preparation of the carrier conjugate and, in addition, may orient the immobilized molecules in a well-defined way on the solid phase. Covalent coupling has been described for peptides via amino groups with disuccinimidyl suberate as coupling agent [25], or via carboxylic acids with EDC as coupling agent [20]. Covalent coupling of oligonucleotides with EDC as coupling agent [26, 27] and the covalent coupling of a model steroid containing carboxyl groups [21] has been described. The present study is the first reported EIA for measuring 25(OH)D₃ with 25(OH)D₃-3-hemisuccinate covalently coupled to the microtiter well.

The 25(OH)D₃-3-hemisuccinate was preactivated with high concentrations of EDC and NHS, whereupon it was diluted in PBS to minimize the concentrations during coupling as described elsewhere [20]. It is important that the EDC and NHS solutions be freshly prepared [21]. In the present study the efficiency of the covalent coupling

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### Table 2. Analytical recovery of 25(OH)D₃ (µg/L).

<table>
<thead>
<tr>
<th>Endogenous Added</th>
<th>Added</th>
<th>Expected</th>
<th>Recovery, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>30.2 (n = 6)</td>
<td>12.5</td>
<td>42.0</td>
<td>42.7</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>55.4</td>
<td>55.2</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>78.9</td>
<td>80.2</td>
</tr>
<tr>
<td>38.7 (n = 3)</td>
<td>10</td>
<td>50.6</td>
<td>48.7</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>61.5</td>
<td>58.7</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>85.7</td>
<td>78.7</td>
</tr>
<tr>
<td>69.4 (n = 6)</td>
<td>12.5</td>
<td>79.4</td>
<td>81.9</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>93.6</td>
<td>94.4</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>116.9</td>
<td>119.4</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td>102 ± 13</td>
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</table>

### Table 3. Cross-reactivity of various vitamin D compounds in the 25(OH)D₃ EIA.

<table>
<thead>
<tr>
<th>Vitamin D metabolite</th>
<th>Cross-reactivity, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D₃</td>
<td>100</td>
</tr>
<tr>
<td>1,25(OH)₂D₃</td>
<td>1000</td>
</tr>
<tr>
<td>24,25(OH)₂D₃</td>
<td>50</td>
</tr>
<tr>
<td>Vitamin D₃</td>
<td>2</td>
</tr>
<tr>
<td>25(OH)D₂</td>
<td>2</td>
</tr>
<tr>
<td>1,25(OH)₂D₂</td>
<td>7</td>
</tr>
</tbody>
</table>

*Cross-reactivity was defined as the reciprocal value of the concentration required to reach 50% displacement, taken relative to that of 25(OH)D₃.*
hemisuccinate was 40 ng/100 mL, the present study the coupling solution of 25(OH)D3-3-hemisuccinate was unique for the coupling of hemisuccinate derivatives. In the present study the coupling efficiency. The observed effect of chloramine T on the coupling of EDC and NHS concentrations and, above all, the addition of chloramine T improved the coupling efficiency. The observed effect of chloramine T on the coupling process is not clearly understood and it may be unique for the coupling of hemisuccinate derivatives. In the present study the coupling solution of 25(OH)D3-3-hemisuccinate was 40 ng/100 µL, and ~48% of the functional groups in the well were occupied. This number is comparable with another study in which ~60% of the functional groups were occupied [20].

All vitamin D assays described so far require some serum pretreatment in which vitamin D is dissociated from the vitamin D binding protein. The pretreatment involves extraction with an organic solvent and often subsequent chromatographic purification. In the present study and in a very few other assays, until now solely RIAs, the extraction procedure was facilitated because it does not include a chromatographic step [18, 19]. The extraction recovery of 25(OH)D3 observed in the present study was comparable with other vitamin D studies [12–14, 28]. We used the same pretreatment of the calibrators and the serum samples, and hence the correction for extract recovery was omitted.

The within-run and between-run variations observed in this EIA were similar to those observed in other vitamin D assays, and so was the accuracy [13, 15, 19, 28]. The antiserum used in the EIA and the RIA was directed towards 1,25(OH)2D3-3-hemisuccinate–BSA. In the EIA this antiserum also reacted with 25(OH)D3, less with 24,25(OH)2D3, and much less with vitamin D2 metabolites and unhydroxylated vitamin D3. The cross-reactivity with 1,25(OH)2D3 and 24,25(OH)2D3 presents no clinical problem, as the circulating concentration of 1,25(OH)2D3 is only about one thousandth of that of 25(OH)D3 [4, 29], and the concentration of 24,25(OH)2D3 is only ~10% compared with 25(OH)D3 [4], thus suggesting a contribution by cross-reacting vitamin D metabolites of <5% of the typically estimated concentration of 25(OH)D3 in serum.

The comparison between the results from the EIA and the more cumbersome RIA showed reasonable accordance. The EIA values were on average 19% higher than the RIA values, the difference being highly significant. On the basis of our experiments of cross-reactivity, only a few percent of this overestimation might be explained by cross-reacting vitamin D metabolites. However, spuriously increased concentrations of 25(OH)D3 have been explained by lipid interference [10], especially in nonchromatographic protein binding assays compared with chromatographic protein binding assays. The present EIA is based on antibodies, which are more specific than the binding protein, and thus should be less affected by nonspecific lipid interference. We found that the lipid interference was negligible in this EIA, and, as yet, we have no explanation for the overestimation of the present EIA as compared with the RIA. However, it is not unusual that different vitamin D assays measure different values [29, 30], implicating the importance of establishing a reference interval for the individual assay.

The main feature of the present EIA was the use of covalent coupling of a hemisuccinate derivative of 25(OH)D3 onto CovaLink microplates. This model of an assay could be adapted for measuring other steroid molecules. This new EIA appeared adequate to estimate 25(OH)D3, and it had several advantages compared with the RIA; the time-consuming liquid scintillation counting was omitted and the results were obtained within the same day of incubation. In conclusion, this EIA can be used as a rapid screening test for vitamin D status and for monitoring a patient’s response to vitamin D3 therapy.

References