Automated Measurement of 25-OH Vitamin D₃ on the Roche Modular E170 Analyzer

Aila Leino,¹,²* Ursula Turpeinen,³ and Pertti Koskinen¹,²

¹ TYKSLAB, the Hospital District of Southwest Finland; ² Department of Clinical Chemistry, University Hospital, Turku, Finland; ³ HUSLAB, Laboratory of Women’s Clinic, Helsinki, Finland; *address correspondence to this author at: Department of Clinical Chemistry, University Hospital of Turku, Kiinamyllynkatu 4-8, FIN-20520 Turku, Finland. Fax +358-2-3133933; e-mail aila.leino@tyks.fi.

BACKGROUND: The first commercial direct automated immunoassay specific for 25-OH vitamin D₃ (25-OH-D₃) was recently introduced for use on Roche Diagnostics immunoassay analyzers. We assessed the analytical performance of the Elecsys 25-OH-D₃ assay on a Roche Modular E 170 analyzer.

METHODS: The Elecsys 25-OH-D₃ assay is a direct electrochemiluminescence immunoassay for human serum or plasma. It is a competitive assay in which the binding protein of vitamin D is inactivated during incubation. The assay employs a polyclonal antibody directed against vitamin 25-OH-D₃. We compared the 25-OH-D₃ assay to assays performed with RIA, HPLC, and liquid chromatography–tandem mass spectrometry (LC-MS/MS).

RESULTS: At concentrations of 48, 76, and 124 nmol/L, within-run CVs were 5.1%, 3.1%, and 7.1% and total CVs were 12.1%, 7.4%, and 10.6%, respectively. A comparison of Elecsys 25-OH vitamin D₃ with RIA yielded the regression equation: Elecsys = 1.114 × RIA – 6.15 (S y/x = 15.7 nmol/L; n = 163). The corresponding equation with HPLC was: Elecsys = 1.077 × HPLC + 5.442 (S y/x = 13.9 nmol/L; n = 67) and with LC-MS/MS: Elecsys = 0.887 × LC-MS/MS + 5.046 (S y/x = 12.4 nmol/L; n = 64). Compared with LC-MS/MS, with the cutoff of 50 nmol/L (deficiency vs normal), approximately 10% of samples were misclassified as normal with RIA and Elecsys. Plasma samples were observed to have markedly higher concentrations than serum samples.

CONCLUSIONS: The Elecsys concentrations of 25-OH-D₃ were in good overall agreement with those determined with LC-MS/MS and RIA. However, large between-method variation was observed in individual patient samples. Use of serum rather than plasma is preferred owing to the higher results observed with plasma samples.

The roles of vitamin D as a key regulator of absorption of calcium and inorganic phosphate from the intestine and as a risk factor for osteoporosis are well established. Emerging data indicate that vitamin D deficiency is a risk factor for many common and serious diseases, including cancers, diabetes, and cardiovascular diseases (1), increasing the importance of vitamin D measurements and creating a need for automation of vitamin D assays, with improved throughput and efficiency.

Quantification of 25-hydroxyvitamin D (25-OH-D)²⁴ in serum is the best indicator of overall vitamin D status (2). The important forms of vitamin D are vitamin D₃ (cholecalciferol) and vitamin D₂ (ergocalciferol). However, the potency of vitamin D₃ is less than one-third that of vitamin D₃, and vitamin D₂ has a shorter duration of action than vitamin D₃ (3).

These 2 forms of vitamin D are delivered to and carried in the blood as a complex with vitamin D–binding protein. Once in the circulation, vitamin D is hydroxylated to vitamin 25-OH-D in the liver. More than 95% of 25-OH-D measurable in serum is typically 25-OH vitamin D₃ (25-OH-D₃), whereas 25-OH vitamin D₂ (25-OH-D₂) reaches measurable concentrations only in patients taking vitamin D₂ supplements (4).

Various manual assays are available for the quantification of circulating 25-OH-D₃ and 25-OH-D₂ that incorporate antivitamin D antibodies for analyte recognition. Several HPLC methods with ultraviolet detection have been described, but their routine use is limited by complex sample preparation requirements (5).

Recently, the first commercial direct automated immunoassay specific for vitamin 25-OH-D₃ has been introduced. This Elecsys 25-OH-D₃ assay (Roche Diagnostics) is a direct electrochemiluminescence immunoassay for human serum or plasma intended for use on Roche immunoassay analyzers. The method follows a competitive assay principle in which the binding protein of vitamin D is inactivated during incubation. The assay employs a polyclonal antibody directed against vitamin 25-OH-D₃. For our evaluation of this method we obtained consecutive samples sent for routine vitamin 25-OH-D₃ analysis. After blood collection the gel

tubes were centrifuged within 1 hour and the sera stored at −20 °C for 1 week until analysis.

The analytical performance of the Elecsys 25-OH-D₃ vitamin assay on a Roche Modular E170 immunoanalyzer was compared with a manual RIA method (25-hydroxyvitamin D¹²⁵I RIA kit, DiaSorin), an HPLC method with ultraviolet detection (6), and a liquid chromatography–tandem mass spectrometry (LC-MS/MS) assay.

In the LC-MS/MS method, we added 20 µL of 0.5 µmol/L deuterium-labeled internal standard 26,27-hexafluorouronium-25-OH-vitamin D₃ (Synthetica AS) in 500 mL/L methanol/water to 0.25 mL of patient or QC serum before extraction. We precipitated the proteins by adding 175 µL of methanol and mixing for 10 s, then performing extraction for 3 min with 1.5 mL hexane in a multitube vortexer. The phases were separated by centrifugation at 2100 g for 5 min, and the upper organic phase was transferred to a conical tube and dried under nitrogen. The residue was dissolved in 250 µL of 800 mL/L methanol/water. Six calibrators containing 5, 10, 25, 50, 100, and 200 nmol/L of 25-OH-D₂ and 25-OH-D₃ (Fluka, Sigma-Aldrich Chemie) were prepared in 800 mL/L methanol/water by dilution of the stock solution (2 g/L) in ethanol. We verified the calibrator concentrations by spectrophotometry with a molar absorption coefficient of 18 200 for both metabolites at 265 nm in ethanol.

We analyzed 25 µL of sample extracts and calibrators on an LC-MS/MS system that included an API 3000 triple quadrupole mass spectrometer (Applied Biosystems) and an Agilent series 1100 HPLC system with a binary pump. Separation was performed on a Sunfire C18 column (2.1 × 50 mm, 3.5 µm; Waters) at 30 °C and a flow rate of 250 µL/min. The mobile phase was a gradient consisting of methanol and water: 0 min, 80% methanol; 2 min, 80% methanol; 2.1 min, 100% methanol; 3.6 min, 100% methanol; and 4 min, 80% methanol. The column was directly connected to the electrospray ionization probe operating at 325 °C.

25-OH-D₂ and 25-OH-D₃, and IS were detected in the multiple-reaction–monitoring mode with the following transitions: 25-OH-D₂, m/z 413.3 to m/z 395.5; 25-OH-D₃, m/z 401.5 to m/z 383.6; and internal standard, m/z 407.5 to m/z 389.6. Data were acquired and processed with the Analyst Software (Ver. 1.4, Sciex). All results were generated in positive-ion mode with the entrance potential at 9 and 10 V, the declustering potential at 48 and 50 V, and the collision cell exit potential at 19 and 21 V. The optimized focusing potentials were set at 350 V and the collision energy potential
at 13 and 12 V as determined by manual tuning for 25-OH-D$_3$ and 25-OH-D$_2$, respectively. Front-end electrospray settings for the MS/MS ionization source were as follows: curtain gas, 6; nebulizer gas, 8; GS2, 7000; CAD, 6; probe temperature 325 °C; and ion spray voltage 5500 V. For all MS/MS experiments, mass calibration and resolution adjustments on both the resolving quadrupoles were optimized by use of a polypropylene glycol solution with an infusion pump. Collisionally activated decomposition MS/MS was performed with nitrogen as collision gas.

The RIA, HPLC, and LC-MS/MS methods measure both forms of vitamin, 25-OH-D$_1$ and 25-OH-D$_3$. With the RIA method the results are reported as total 25-OH-D.

The agreement of Elecsys 25-OH-D$_3$ vitamin assay with the comparison methods is shown in Fig. 1.

The correlation between methods was (numbers in parentheses are SEs of slopes and intercepts):

- Elecsys (nmol/L) = 1.114 (0.074) × DiaSorin RIA (nmol/L)
  − 6.15 (4.10) nmol/L,
  $S_{y/x} = 15.7$ nmol/L, $r = 0.836,$
  $n = 163$ (Eq. A);

- Elecsys (nmol/L) = 1.077 (0.108) × HPLC (nmol/L)
  + 5.442 (4.986) nmol/L,
  $S_{y/x} = 13.9$ nmol/L, $r = 0.808,$
  $n = 67$ (Eq. B);

- Elecsys (nmol/L) = 0.887 (0.094) × LC-MS/MS (nmol/L)
  + 5.046 (5.225) nmol/L,
  $S_{y/x} = 12.4$ nmol/L, $r = 0.8157,$
  $n = 64$ (Eq. C).

In the Bland–Altman plots the means (SDs) of paired differences were 0.7 (14.5) nmol/L, 9.4 (13.5) nmol/L, and −1.6 (13.4) nmol/L, respectively. In all samples the observed 25-OH-D$_3$ concentrations were <10 nmol/L with the HPLC and LC-MS/MS methods. The mean (SD) concentrations of vitamin 25-OH-D$_3$ in 20 serum and plasma samples drawn simultaneously and measured in the same run with the Elecsys assay were 69.2 (14.3) nmol/L, 81.7 (13.8) nmol/L, and 73.9 (13.5) nmol/L in serum, lithium heparin plasma, and EDTA plasma, respectively. The difference of concentrations between serum and lithium heparin plasma was statistically significant ($P < 0.001$). With LC-MS/MS, however, no differences were observed for these 3 specimen types.

We evaluated the imprecision of the Elecsys 25-OH-D$_3$ assay with serum pools and commercial controls. The within-run CVs were 5.1%, 3.1%, and 7.1% at concentrations of 48, 76, and 124 nmol/L. Total CVs were 12.1%, 7.4%, and 10.6%, respectively. The sample requirement was 35 μL, the stated measuring range 10–250 nmol/L and the maximum theoretical throughput with Modular E was 170 samples/h. According to the manufacturer, the use of both serum and plasma are acceptable. However, we recommend use of serum samples owing to the higher results we observed with plasma samples.

The 25-OH-D$_3$ concentrations we measured with the Roche Elecsys were in good overall agreement with those measured with LC-MS/MS and RIA, with the means of paired differences being only −1.6 nmol/L and 0.7 nmol/L, respectively. Contrary to the Elecsys assay, which is specific for 25-OH-D$_3$, the Diasorin RIA measures 25-OH-D$_1$ and 25-OH-D$_3$ on an equimolar basis. Therefore, in individuals who receive ergocalciferol supplementation, the Elecsys assay may underestimate the vitamin D status, unlike the other assays in this study. However, in Finland cholecalciferol preparations are most commonly used for vitamin D supplementation in adults.

Although the Elecsys concentrations of 25-OH-D$_3$ were in good overall agreement with those determined with LC-MS/MS and RIA, large between-method differences were observed in some individual samples. Compared with LC-MS/MS, with the cutoff of 50 nmol/L used to classify deficient vs normal results (1), approximately 10% of samples were misclassified as normal with RIA and Elecsys. This result raises concern, especially of the spuriously high 25-OH-D$_3$ results observed near the cutoff of optimal vitamin D concentrations (50–80 nmol/L) (2) with RIA and Elecsys. Furthermore, our results are in accordance with recent observations of Beastall and Rainbow (7), who reported high all-method mean CV for D-vitamin methods in an external QC survey.

We conclude that the Roche Elecsys 25-OH-D$_3$ assay provides the benefit of automation and greater convenience, potentially improving laboratory workflow and efficiency. We believe that the clinical value of the assay would further be improved by improving the analytical sensitivity at the lower end of the measuring range and diminishing the matrix effects that lead to variation in individual patient samples.
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References


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