# 상품화된 3개 회사의 비타민 D 측정 방법의 비교
## Comparison of Three Commercially Available Assays for Measurement of Vitamin D

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**Background:** We evaluated three commercially available vitamin D assays to evaluate and compare the correlation and accuracy among them.
**Methods:** Vitamin D was measured in 71 patient samples using the Architect 25-OH vitamin D assay (Abbott), the ADVIA Centaur vitamin D total assay (Siemens), and the LIAISON 25 OH vitamin D total assay (Diasorin). The evaluation made use of both patient samples and standard reference material, SRM 972. To analyze correlations and differences, Pearson's correlation coefficients and paired sample $t$-tests were performed.
**Results:** Correlations among the three evaluated assays showed strong positive linear relationships (correlations among Siemens and DiaSorin, DiaSorin and Abbott, Abbott and Siemens: r = 0.935, r = 0.927, r = 0.909, respectively). Mean (SD) vitamin D values on Siemens, Abbott, and DiaSorin assays in the 71 patient samples were 23.09 (10.41), 16.75 (11.26), and 16.76 (9.32), respectively. Results for the Siemens assay were significantly different from the other two methods ($P < 0.001$). Target values for SRM 972 level 1, 2, 3, and 4 were 23.9, 14.0, 44.9, and 35.4, respectively. The Abbott, Siemens, and Diasorin assay values were closest to the target values in level 1, levels 2 and 3, and level 4, respectively.
**Conclusions:** Correlations among vitamin D assays were good; however, the mean values of the Siemens assay were significantly higher than those of DiaSorin or Abbott. We found significant differences in vitamin D levels and discrepancies between patient samples and SRM 972 samples, which should be considered during use in a clinical setting.

**Key Words:** Vitamin D, 25-Hydroxyvitamin D, Immunoassay

## INTRODUCTION

Vitamin D is recognized for its central role in the maintenance of calcium and phosphate homeostasis, both of which are essential for bone health [1]. Increased recognition of the relationship between vitamin D and a variety of diseases, such as diabetes, autoimmune disease, musculoskeletal disease, and various cancers, has led to a massive rise in the need for vitamin D testing [2-6].

Humans have two types of vitamin D, endogenously derived vitamin $D_3$ and $D_2$ that is derived from the diet [7]. Vitamin D is converted to 25-hydroxyvitamin D (25-OH-D) in the liver, and a fraction of circulating 25-OH-D is converted to its active metabolite, 1-25-dihydroxyvitamin D (1-25-$(OH)_2$-D), in the kidney [8]. These metabolites are bound to vitamin D binding protein (80–90%) and albumin (10–20%). Only a very small fraction (0.02–0.05% of 25-OH-D and 0.2–0.6% of total 1-25-$(OH)_2$-D) remains unbound. Although 1-25-$(OH)_2$-D is the biologically active form, it is widely accepted that serum 25-OH-D is the best single marker for vitamin D status [9, 10].

Complex regulatory mechanisms control metabolism, with recent clinical studies suggesting there is a narrow physiological range of serum vitamin D levels in which metabolic functions are optimized [11, 12]. Levels above or below this natural vitamin D homeostasis are associated with increased mortality. Although optimal levels of 25-OH-D in the body remain unknown, a level >30 ng/mL is recommended [6, 13].

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Laboratories require a reliable, automated assay to cope with the increased demand for accurate vitamin D status measurement. Currently, considerable discrepancies between laboratories, as well as differences in analytical methods, have been consistently reported [10, 14, 15]. The aim of this study was thus to evaluate and compare the correlation and accuracy among three commercially available vitamin D assays made by Siemens, Abbott, and DiaSorin.

## MATERIALS AND METHODS

### 1. Specimens

This study was approved by the independent Institutional Review Board of Kosin University Gospel Hospital (KUGH MDIRB 11-58). The study was exempt from informed consent since residual serum samples were obtained from patients as part of routine testing for total vitamin D in the clinical laboratory. A total of 71 patient samples, referred from various departments for routine blood testing, were used. Patients were on average 53.3 yr old (1–74), with an M:F ratio of 0.4 (22:49).

### 2. Vitamin D assays

The Architect 25-OH vitamin D assay from Abbott (Abbott vit D; Abbott Laboratories, Abbott Park, IL, USA), the ADVIA Centaur vitamin D total assay from Siemens (Siemens vit D; Siemens Healthcare Diagnostics, Deerfield Road, IL, USA), and the LIAISON 25 OH vitamin D total assay from DiaSorin (DiaSorin vit D; DiaSorin Inc., Northwestern Avenue, MN, USA) were used to measure vitamin D according to the respective manufacturer's instructions.

### 3. Standard reference material

Standard reference material (SRM) 972 (National Institute of Standards & Technology, Gaithersburg, MD, USA) was used to assess the accuracy of each assay. A unit of SRM 972 consists of four vials (level 1 through 4) of frozen serum with different 25-OH-D concentrations. Level 1 of SRM 972 was prepared from normal human serum and was not altered. Level 2 was prepared by diluting level 1 with horse serum to achieve a lower 25-OH-D concentration. Levels 3 and 4 contain normal human serum fortified with 25-OH-$D_2$ and 3-epi-25-OH-$D_3$, respectively.

### 4. Vitamin D measurement

Vitamin D measurements by the Abbott vit D, Siemens vit D, and DiaSorin vit D kits were analyzed using Architect (Abbott, USA), ADVIA Centaur XP (Siemens, USA) and LIAISON (DiaSorin, USA) analyzers, respectively.

### 5. Evaluation design

Patient samples were divided into three tubes for the measurement of vitamin D with the Siemens vit D, Abbott vit D and DiaSorin vit D kits, respectively. The mean, standard deviation (SD), coefficient of variation (CV), and association among them were calculated.

We also observed vitamin D distribution in the 71 patient samples using each of the three vitamin D assays. We compared the three assays via linear regression and Bland-Altman plots. Differences in values between paired vitamin D assays were analyzed and the statistical significance of these differences was determined.

To evaluate accuracy, each of the SRM 972 levels was tested in triplicate. Reference materials were treated in the same manner as patient specimens and the assays run according to the respective manufacturer's instructions. The mean, SD, CV, and percentage of measured value relative to target value (M/T) was calculated for each level.

### 6. Statistical methods

To establish the correlation among vitamin D levels measured by each of the three commercial vitamin D assays, Pearson's correlation coefficients and mean bias were calculated and evaluated using paired sample $t$-tests. SPSS Statistics 17.0 (IBM SPSS Inc., Chicago, IL, USA) was used for statistical analyses, and differences were considered statistically significant at $P < 0.05$.

## RESULTS

### 1. Comparison of Siemens, Abbott, and DiaSorin vitamin D assays

Vitamin D concentrations were established to range from 2.4 to 70.1 ng/mL (Fig. 1). Siemens vit D, Abbott vit D, and DiaSorin vit D showed strong positive linear relationships (correlations between DiaSorin vit D and Abbott vit D (Fig. 1A); Siemens vit D and DiaSorin vit D (Fig. 1C); Abbott vit D and Siemens vit D (Fig. 1E): r = 0.927, $P < 0.001$; r = 0.935, $P < 0.001$; r = 0.909, $P < 0.001$, respec-
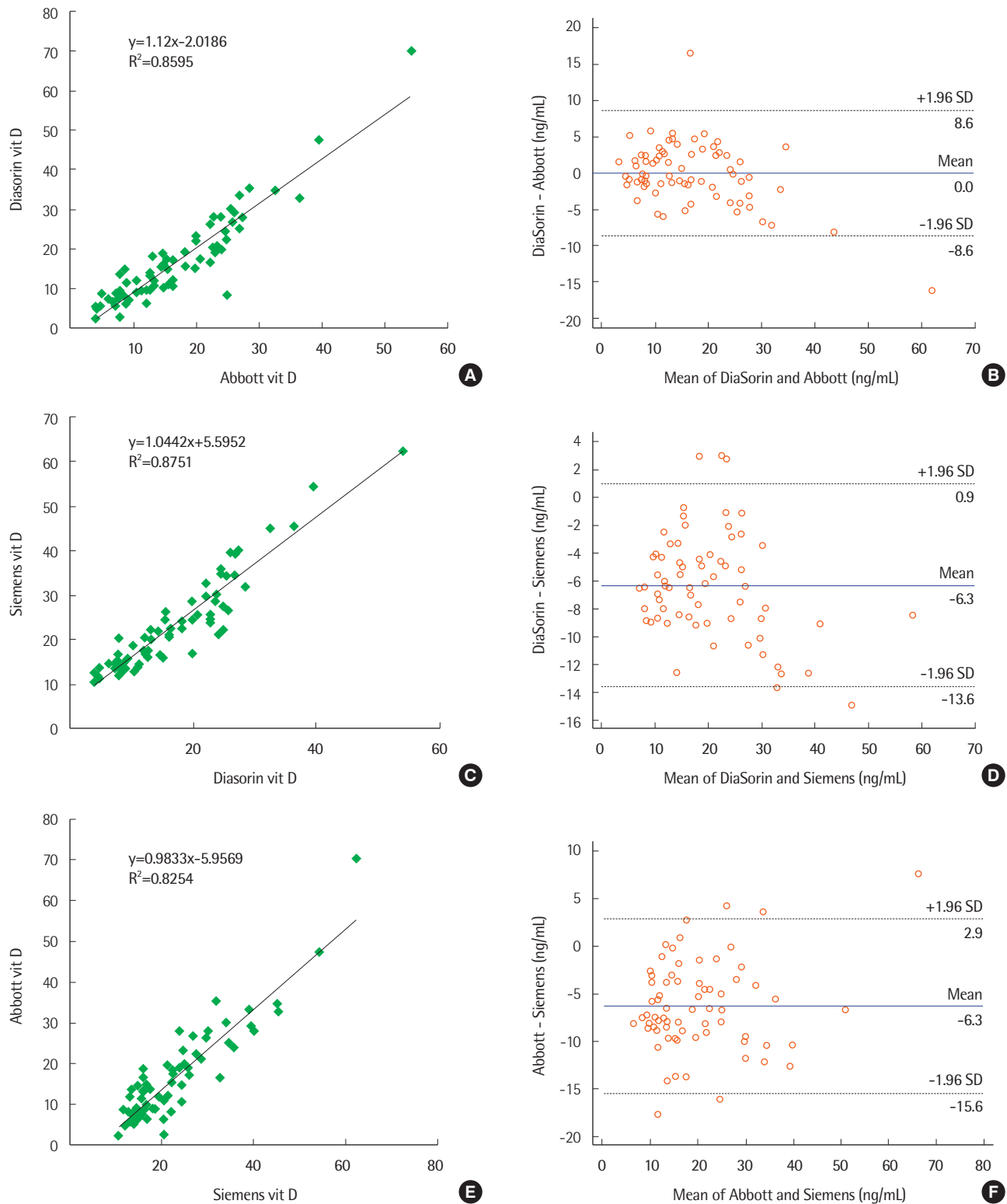
Fig. 1. Linear regression and Bland-Altman analyses of immunoassays. (A) and (B), Abbott and DiaSorin; (C) and (D), DiaSorin and Siemens; and (E) and (F), Abbott and Siemens.
Abbreviations: Abbott, Architect 25-OH vitamin D assay of Abbott; DiaSorin, LIAISON 25 OH vitamin D total assay of DiaSorin; Siemens, ADVIA Centaur vitamin D total assay of Siemens.
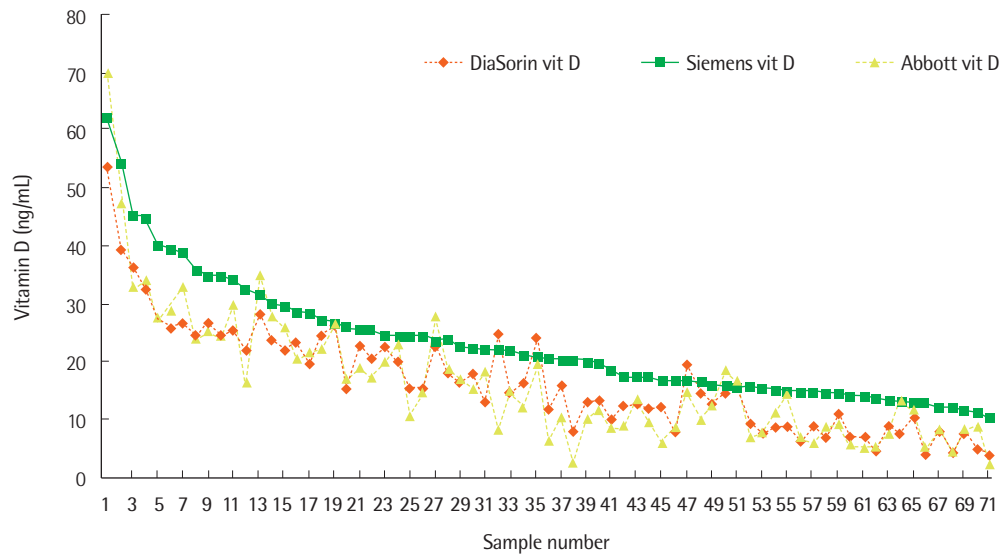
Fig. 2. Distribution of vitamin D levels of all samples by Siemens, Abbott, and DiaSorin. The mean (SD) of Siemens, Abbott, and DiaSorin assays was 23.09 (10.41), 16.75 (11.26), and 16.76 (9.32), respectively. Siemens (■), Abbott (▲), and DiaSorin (♦) indicate the ADVIA Centaur vitamin D total assay of Siemens, the Architect 25-OH vitamin D assay of Abbott, and the LIAISON 25 OH vitamin D total assay of DiaSorin, respectively.

Table 1. Paired sample *t*-tests among Siemens, Abbott, and DiaSorin vitamin D assays

| Paired group | Mean | SD | 95% Confidence Interval | | *P*-value |
| | | | Lower | Upper | |
|---|---|---|---|---|---|
| DiaSorin – Abbott | 0.00704 | 4.36700 | -1.02661 | 1.04069 | 0.989 |
| DiaSorin – Siemens | -6.33577 | 3.70147 | -7.21190 | -5.45965 | <0.001 |
| Abbott – Siemens | -6.34282 | 4.70884 | -7.45738 | -5.22825 | <0.001 |

The difference between the paired assays is smaller between DiaSorin and Abbott compared to that between Siemens and each of the other two assays, and the DiaSorin – Abbott difference is not significant. In contrast, the differences between DiaSorin and Siemens and between Abbott and Siemens assays were significant.

tively). The mean bias between DiaSorin vit D and Abbott vit D (Fig. 1B), Siemens vit D and DiaSorin vit D (Fig. 1D), and Abbott vit D and Siemens vit D (Fig. 1F) was +0.007 ng/mL, -6.336 ng/mL and -6.343 ng/mL, respectively. The correlation between Siemens vit D and DiaSorin vit D was the strongest among the tested assays.

## 2. Differences in vitamin D values among the Siemens, Abbott, and DiaSorin vitamin D assays

Mean (SD) vitamin D values for the Siemens, Abbott, and DiaSorin assays in the 71 patient samples were 23.09 (10.41), 16.75 (11.26), and 16.76 (9.32), respectively. Distribution of the vitamin D values, in the 71 patient samples, showed a tendency for the Siemens assay to produce higher values than those from the other two assays (Fig. 2). In the paired *t*-test, results from the Siemens vit D assay were considered significantly different from those of the DiaSorin vit D and Abbott vit D assays (*P*<0.001) (Table 1).

Table 2. Accuracy of the DiaSorin, Abbott, and Siemens vitamin D assays

| SRM 972 (Target value) | | Siemens | Abbott | DiaSorin |
|---|---|---|---|---|
| Level 1 (23.9) | Mean (ng/mL) | 14.94 | 19.17 | 17.67 |
| | SD | 1.46 | 0.55 | 0.42 |
| | CV (%) | 9.76 | 2.87 | 2.36 |
| Level 2 (14.0) | Mean (ng/mL) | 14.33 | 14.43 | 17.50 |
| | SD | 1.69 | 0.12 | 1.25 |
| | CV (%) | 11.76 | 0.80 | 7.16 |
| Level 3 (44.9) | Mean (ng/mL) | 40.66 | 20.33 | 27.40 |
| | SD | 2.20 | 0.45 | 0.17 |
| | CV (%) | 5.40 | 2.22 | 0.63 |
| Level 4 (35.4) | Mean (ng/mL) | 21.24 | 23.97 | 29.30 |
| | SD | 1.67 | 0.42 | 0.40 |
| | CV (%) | 7.88 | 1.74 | 1.37 |

Siemens, Abbott, and DiaSorin in the table indicate the ADVIA Centaur vitamin D total assay of Siemens, the Architect 25-OH vitamin D assay of Abbott, and the LIAISON 25 OH vitamin D total assay of DiaSorin, respectively.

## 3. Accuracy analysis

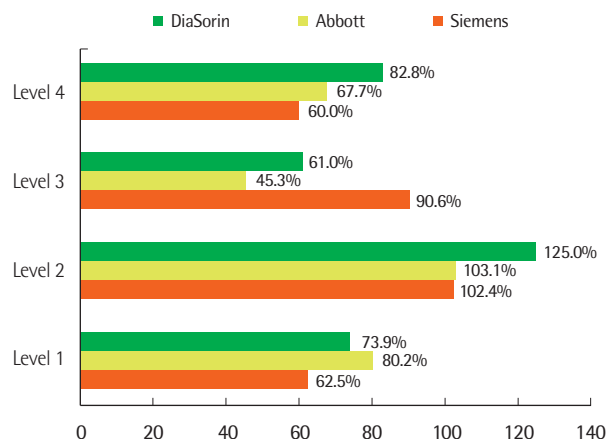Target values for SRM 972 levels 1, 2, 3, and 4 were 23.9, 14.0, 44.9,

Fig. 3. Measured values relative to target values for vitamin D. Target values for SRM 972 levels 1, 2, 3, and 4 were 23.9, 14.0, 44.9, and 35.4, respectively. Siemens (■), Abbott (■), and DiaSorin (■) indicate the ADVIA Centaur vitamin D total assay of Siemens, the Architect 25-OH vitamin D assay of Abbott, and the LIAISON 25 OH vitamin D total assay of DiaSorin, respectively.

and 35.4, respectively. Measured values from the Abbott vit D kit were closest to the level 1 target value (value, M/T: 19.17, 80.2%); the Siemens vit D kit values were closest to levels 2 and 3 (L2: 14.33, 102.4%; L3: 40.66, 90.6%); and the DiaSorin vit D kit values were closest to level 4 (29.30, 82.8%). CVs for Siemens vit D were higher than those for Abbott vit D and DiaSorin vit D for all SRM 972 levels (Table 2, Fig. 3). The measured value, as a percentage of the target value, was larger for level 2 than for any of the other levels (Fig. 3).

## DISCUSSION

There are several issues that must be considered when measuring vitamin D status. First, there is controversy surrounding the bioequivalence of vitamin $D_2$ and $D_3$. In one report vitamin $D_3$ is suggested to be more potent than $D_2$ in raising and maintaining the serum 25-OH-D concentration [16], whereas another suggests that $D_2$ and $D_3$ maintain 25-OH-D status equally [17]. Furthermore, immunoassays should react equally to 25-OH-$D_2$ and 25-OH-$D_3$, yet several reports suggest there are differences in the reactivity to these metabolites to various manufacturer's immunoassay kits [18]. Second, 25-OH-D cannot be accurately measured unless it is separated from its specific binding protein. Competition between organic solvents and vitamin D binding protein (DBP) in the plasma makes commercial assays prone to inaccuracies [19]. Another challenge, inherent to vitamin D immunoassays, is the discrepancy

within and between immunoassays due to cross-reactivity with other vitamin D metabolites or interference by polyclonal antibodies [20]. Confounding matrix substances, such as lipids, were also suggested to cause significant differences in the 25-OH-D levels between various assays [21]. The lipophilic nature of 25-OH-D also affects the reactions between it and the binding agent used in the assay sample and standard [22]. Although the biological activity of 25-OH-D in humans is unlikely to be significant enough to affect the overall vitamin D concentration, they are still potential sources of measurement bias.

According to the manufacturer, the Abbott vit D assay was designed to have a correlation coefficient for serum samples equal to or higher than 0.80 when evaluated against the DiaSorin vit D kit [23]. The Siemens vit D assay was also previously evaluated by liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS), using 580 samples in the range of 4 to 150 ng/mL vitamin D [24]. From these results, it can be assumed that the difference between the Abbott vit D and DiaSorin vit D assay means is not significant, but there must be a significant difference between the Siemens vit D and other two assays. Although vitamin D values from the three assays were strongly, positively correlated (Fig. 1), vitamin D levels in 8 of the 71 patient samples measured by the Siemens kit were lower than those from the Abbott or DiaSorin kits, and higher in the remaining 63 samples (Fig. 2). Overall, the vitamin D levels measured by the Siemens vit D assay were significantly higher when compared to the other two methods (Table 1). However, values obtained using the Siemens vit D assay for three of the SRM 972 levels (levels 1, 2, and 4) were lower than those obtained using the other two methods (Table 2, Fig. 3). There were different trends in the results depending on the samples used for analysis.

Heijboer et al. [19] reported an inverse relationship between DBP concentration and the results of the isotope dilution/online solid-phase extraction liquid chromatography/tandem mass spectrometry 25-OH-D reference method; incomplete release of 25-OH-D from the DBP was suggested as a potential source of variability for automated immunoassays [19]. However, Freeman et al. [25] reported that the Siemens vit D assay demonstrated acceptable performance when compared with an LC-MS/MS method regardless of the amount of DBP [25]. This suggests that the amount of DBP may not have caused the differences in 25-OH-D concentrations observed between the Korean population and the SRM

972 samples used in this study. Another factor that may cause such differences in 25-OH-D concentrations is DBP polymorphisms [26]. By comparison to white-skinned populations, black and yellowish skin type populations have a relatively high frequency of the GC1F allele [27]. The GC genotype of DBP may be related to a susceptibility to low 25-OH-D levels [28], with the GC1F variant most frequently observed in Korean populations [29]. Genetic differences in DBP in the Korean population may have caused the observed discrepancy in the results from SRM 972 samples used in this study. However, the possibility of other interferences from different metabolites or isomers or a matrix effect should also be considered.

When we measured the standard reference material, SRM 972, and calculated the differences between the assay producing the value closest to the target value and the other two values for each level, the difference was greatest at level 3 (range of differences in levels 1, 2, 3, and 4 among the three assays: 6.3-17.7, 0.7-22.6, 29.6-45.3 and 15.1-22.8, respectively). Discrepancies among these assays might be the result of the increased metabolite concentrations around level 3 (44.9 ng/mL). Moon et al. [30] compared vitamin D values obtained via the Siemens vit D, Diasorin vit D, and Roche assays in a Korean population; correlation between Siemens and Diasorin was strongest (R=0.9390), while the difference in % bias between Siemens vit D and Diasorin vit D was greatest at SRM 972 level 3 than at any other level, as was similarly seen in our results. The percent difference between Siemens vit D and Diasorin vit D using three fresh frozen samples (College of American Pathologists [CAP] Accuracy-Based Vitamin D [ABVD] 2013 survey) also showed that the % difference of Diasorin vit D was much lower than that of Siemens vit D for ABVD1 (target concentration=41.5 ng/mL, similar to level 3 SRM 972) [31], which further supports our results. Although the relationship cannot be clearly addressed in this study, the ability of the Siemens and DiaSorin vit D assays to detect vitamin D may be related to the presence of 25-OH-D$_2$ and 3-epi-25-OH-D$_3$, respectively. Considering that vitamin D is usually measured to establish a deficiency in the body and that the reference range for deficiency is lower than 10 ng/mL, a test method with more accuracy in the low range would be useful in clinical practice.

As shown in Table 2, the CVs of Siemens vit D were also larger than those of the other two automated vit D assays (Siemens vs. Abbot vs. DiaSorin; 5.40-11.76% vs. 0.80-2.87% vs. 0.63-7.16%).

The CVs of Siemens vit D were also larger than those of other automated vit D assays in previous studies [32]. Hsu et al. [33] reported that the Siemens vit D assay showed a CV from 11.0% to 16.3% over the assay range from 7.0 to 34.6 ng/mL compared to the DiaSorin vit D assay, which demonstrated a total CV from 5.5% to 10.0% [33]. Chen et al. [34] reported that the Siemens vit D assays demonstrated a maximum total CV of 14.1%, while the Roche E170 vit D assay showed a value of 5.9%. Although the Siemens vit D assay demonstrated acceptable imprecision in previous studies, the large CV of the Siemens vit D assay herein reported needs to be improved to be comparable to those of other automated immunoassays. There are continuous debates regarding the best method for measuring 25-OH-D in routine clinical laboratories [31]. Although differing values of vitamin D in our data might be recognized as clinically negligible, our study highlights the fact that significant differences in current vitamin D immunoassays exist (depending on the manufacturer), which could potentially impact clinical practice.

One limitation of our analysis is that we were unable to compare our results using liquid chromatography–tandem mass spectrometry (LC-MS), which is considered the gold standard [35]. We also did not analyze the allele manifestations of DBP in the samples. Since SRM 972 ensures an accurate and comparable measure of vitamin D metabolites in human serum, the results from this analysis should be considered reliable. However, the possibility of an influence on serum 25-OH-D levels due to race, age, ultraviolet B exposure, menopause, or seasonal differences cannot be completely excluded [36, 37]. The new Abbott 25-OH Vitamin D assay released in 2016 in Korea was not analyzed in this study.

In conclusion, correlations among Siemens, DiaSorin, and Abbott vit D assays were good; however, the mean values of the Siemens vit D assay were significantly higher than those of DiaSorin and Abbott. Although this difference might be clinically negligible, we found significant differences in vitamin D levels depending on the manufacturer, which should be considered in clinical practice. Further studies are needed to clarify the cause of the discrepancies observed between Korean patient samples and the SRM 972 samples by considering factors such as genetic differences in DBP as well as interference from other metabolites or isomers, or from a matrix effect. Clinicians thus need to recognize the limitations of the current assays when planning clinical and public health applications.

## 요 약

**배경:** 현재 상품화된 3개 회사 비타민 D 검사법의 상관성과 정확도를 비교평가 하였다.

**방법:** Architect 25-OH vitamin D assay (Abbott), ADVIA Centaur vitamin D total assay (Siemens) 그리고 LIAISON 25 OH vitamin D total assay (Diasorin)의 비교를 위하여 71명의 환자검체로 vitamin D를 측정하였다. 환자검체와 표준물질인 SRM 972를 사용하여 결과값의 상관성과 통계학적 차이를 분석하기 위해 피어슨의 상관계수와 대응표본 $t$-검정을 각각 이용하였다.

**결과:** 비교 평가한 3개 회사 검사법들의 상관성은 강한 양의 비례 상관성을 보였다. Siemens와DiaSorin, DiaSorin과 Abbott, 그리고 Abbott와 Siemens의 상관성은 각각 다음과 같았다: r=0.935, r=0.927, r=0.909. 71명의 환자를 대상으로 한 Siemens, Abbott, 그리고 DiaSorin 검사의 평균(표준편차)은 각각 23.09 (10.41), 16.75 (11.26), 그리고 16.76 (9.32)였다. Siemens의 결과는 다른 두 회사의 검사법과는 통계학적으로 의미있는 차이가 있었다($P<0.001$). SRM 972의 level 1, 2, 3, 그리고 level 4에서의 목표치는 각각 23.9, 14.0, 44.9, 그리고 35.4였다. Abbott, Siemens 그리고 Diasorin 검사법은 각각 level 1, level 2와 level 3, 그리고 level 4에서 각각 목표치와 가장 근사한 값을 보였다.

**결론:** 검사법 간의 상관성은 좋았으나 Siemens 검사법에 의한 검사 결과값들의 평균 값이 DiaSorin과 Abbott 검사법에 비해 의미있게 높았다. Vitamin D 검사 방법 간의 결과값의 의미있는 차이가 존재하며, 이러한 결과값의 양상이 환자 검체를 사용한 경우와 SRM 972을 검사한 경우 달랐으며, 이러한 점들은 임상에서 실제로 비타민 D 검사를 시행하는 경우 고려되어야 한다.

## REFERENCES

1. Audran M and Kumar R. The physiology and pathophysiology of vitamin D. Mayo Clin Proc 1985;60:851-66.

2. Chlebowski RT. Vitamin D and breast cancer: interpreting current evidence. Breast Cancer Res 2011;13:217.

3. Chiang KC, Yeh CN, Chen MF, Chen TC. Hepatocellular carcinoma and vitamin D: a review. J Gastroenterol Hepatol 2011;26:1597-603.

4. Woloszynska-Read A, Johnson CS, Trump DL. Vitamin D and cancer: clinical aspects. Best Pract Res Clin Endocrinol Metab 2011;25:605-15.

5. Choi HS, Kim KA, Lim CY, Rhee SY, Hwang YC, Kim KM, et al. Low serum vitamin D is associated with high risk of diabetes in Korean adults. J Nutr 2011;141:1524-8.

6. Holick MF. Vitamin D deficiency. N Engl J Med 2007;357:266-81.

7. Nair R and Maseeh A. Vitamin D: The "sunshine" vitamin. J Pharmacol Pharmacother 2012;3:118-26.

8. DeLuca HF. Overview of general physiologic features and functions of vitamin D. Am J Clin Nutr 2004;80:1689S-96S.

9. Dawson-Hughes B, Heaney RP, Holick MF, Lips P, Meunier PJ, Vieth R. Estimates of optimal vitamin D status. Osteoporos Int 2005;16:713-6.

10. Holick MF. Vitamin D status: measurement, interpretation, and clinical application. Ann Epidemiol 2009;19:73-8.

11. Hsu JJ, Tintut Y, Demer LL. Vitamin D and osteogenic differentiation in the artery wall. Clin J Am Soc Nephrol 2008;3:1542-7.

12. Medici D, Razzaque MS, Deluca S, Rector TL, Hou B, Kang K, et al. FGF-23-Klotho signaling stimulates proliferation and prevents vitamin D-induced apoptosis. J Cell Biol 2008;182:459-65.

13. Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. Am J Clin Nutr 2006;84:18-28.

14. Carter GD, Carter R, Jones J, Berry J. How accurate are assays for 25-hydroxyvitamin D? Data from the international vitamin D external quality assessment scheme. Clin Chem 2004;50:2195-7.

15. Farrell C, Soldo J, Williams P, Herrmann M. 25-Hydroxyvitamin D testing: challenging the performance of current automated immunoassays. Clin Chem Lab Med 2012;50:1953-63.

16. Heaney RP, Recker RR, Grote J, Horst RL, Armas LA. Vitamin D(3) is more potent than vitamin D(2) in humans. J Clin Endocrinol Metab 2011;96:E447-52.

17. Holick MF, Biancuzzo RM, Chen TC, Klein EK, Young A, Bibuld D, et al. Vitamin D2 is as effective as vitamin D3 in maintaining circulating concentrations of 25-hydroxyvitamin D. J Clin Endocrinol Metab 2008; 93:677-81.

18. Yu S, Cheng X, Fang H, Zhang R, Han J, Qin X, et al. 25OHD analogues and vacuum blood collection tubes dramatically affect the accuracy of automated immunoassays. Sci Rep 2015;5:14636.

19. Heijboer AC, Blankenstein MA, Kema IP, Buijs MM. Accuracy of 6 routine 25-hydroxyvitamin D assays: influence of vitamin D binding protein concentration. Clin Chem 2012;58:543-8.

20. Lee JH, Choi JH, Kweon OJ, Park AJ. Discrepancy between vitamin D total immunoassays due to various cross-reactivities. J Bone Metab 2015;22:107-12.

21. Enko D, Fridrich L, Rezanka E, Stolba R, Ernst J, Wendler I, et al. 25-hydroxy-Vitamin D status: limitations in comparison and clinical interpretation of serum-levels across different assay methods. Clin Lab 2014; 60:1541-50.

22. Wallace AM, Gibson S, de la Hunty A, Lamberg-Allardt C, Ashwell M. Measurement of 25-hydroxyvitamin D in the clinical laboratory: current procedures, performance characteristics and limitations. Steroids 2010;75:477-88.

23. Abbott ARCHITECT® 25-OH Vitamin D Assay [directional insert 3L52]. Abbott Laboratories AP, IL, USA (2010). Abbott Labratories; Abbott Park, IL, USA (2010).

24. Siemens ADVIA Centaur® ACXVDTVAdiERA, 2013-07] Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA (2013).

25. Freeman J, Wilson K, Spears R, Shalhoub V, Sibley P. Influence of vitamin D binding protein on accuracy of 25-hydroxyvitamin D measurement using the ADVIA Centaur Vitamin D Total Assay. Int J Endocrinol 2014;2014:691679.

26. Sinotte M, Diorio C, Berube S, Pollak M, Brisson J. Genetic polymorphisms of the vitamin D binding protein and plasma concentrations of 25-hydroxyvitamin D in premenopausal women. Am J Clin Nutr 2009; 89:634-40.

27. Kamboh MI, Ferrell RE. Ethnic variation in vitamin D-binding protein (GC): a review of isoelectric focusing studies in human populations. Hum Genet 1986;72:281-93.

28. Santos BR, Mascarenhas LP, Boguszewski MC, Spritzer PM. Variations in the vitamin D-binding protein (DBP) gene are related to lower 25-hydroxyvitamin D levels in healthy girls: a cross-sectional study. Horm Res Paediatr 2013;79:162-8.

29. Park Y, Kim YS, Kang YA, Shin JH, Oh YM, Seo JB, et al. Relationship between vitamin D-binding protein polymorphisms and blood vitamin D level in Korean patients with COPD. Int J Chron Obstruct Pulmon Dis 2016;11:731-8.

30. Moon HW, Cho JH, Hur M, Song J, Oh GY, Park CM, et al. Comparison of four current 25-hydroxyvitamin D assays. Clin Biochem 2012;45:326-30.

31. Wyness SP and Straseski JA. Performance characteristics of six automated 25-hydroxyvitamin D assays: Mind your 3s and 2s. Clin Biochem 2015;48:1089-96.

32. Li L, Zeng Q, Yuan J, Xie Z. Performance evaluation of two immunoassays for 25-hydroxyvitamin D. J Clin Biochem Nutr 2016;58:186-92.

33. Hsu SA, Soldo J, Gupta M. Evaluation of two automated immunoassays for 25-OH vitamin D: comparison against LC-MS/MS. J Steroid Biochem Mol Biol 2013;136:139-45.

34. Chen Y, Kinney L, Bozovic A, Smith H, Tarr H, Diamandis EP, et al. Performance evaluation of Siemens ADVIA Centaur and Roche MODULAR Analytics E170 Total 25-OH Vitamin D assays. Clin Biochem 2012;45:1485-90.

35. Saenger AK, Laha TJ, Bremner DE, Sadrzadeh SM. Quantification of serum 25-hydroxyvitamin D(2) and D(3) using HPLC-tandem mass spectrometry and examination of reference intervals for diagnosis of vitamin D deficiency. Am J Clin Pathol 2006;125:914-20.

36. Bikle DD, Gee E, Halloran B, Haddad JG. Free 1,25-dihydroxyvitamin D levels in serum from normal subjects, pregnant subjects, and subjects with liver disease. J Clin Invest 1984;74:1966-71.

37. Harris SS and Dawson-Hughes B. Seasonal changes in plasma 25-hydroxyvitamin D concentrations of young American black and white women. Am J Clin Nutr 1998;67:1232-6.