Humans have two types of vitamin D, endogenously derived vitamin D\textsubscript{3} and D\textsubscript{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)	extsubscript{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)	extsubscript{2}-D) remains unbound. Although 1-25-(OH)	extsubscript{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].
Labs require a reliable, automated assay to cope with the increased demand for accurate vitamin D status measurement. Currently, considerable discrepancies between labs, 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.
Table 2. Accuracy of the DiaSorin, Abbott, and Siemens vitamin D assays

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

Siemens, Abbott, and DiaSorin in the table indicate the ADVA 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, respectively. 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.
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 1, 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 from the Abbott or DiaSorin kits, and higher in the remaining 63 samples (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 (R² = 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-D3 and 3-epi-25-OH-D3, 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 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 바이타민 D 검사법의 상관관계 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 환자검제로 vitamin D를 측정하였다. 환자검체의 표준물질인 SRM 972를 사용하여 결과값의 상관성과 통계학적 차이를 분석하기 위해 피어슨의 상관계수와 대응표본 t-검정을 각각 이용하였다.

결과: 비교 평가한 3개 회사 검사법들의 상관관계는 강한 양의 비례 상관관계를 보였다. Siemens의DiaSorin, DiaSorin과 Abbott, 그리고 Abbott와 Siemens의 상관성은 각각 다음과 같았다: r=0.935, r=0.909. 71명의 환자의 대상으로 한 Siemens, Abbott, 그리고 DiaSorin 검사는의 평균(표준편차)은 각각 23.09 (10.41), 16.75 (11.26), 그리고 16.76 (9.32)었다. Siemens의 결과는 다른 두 회사의 검사법들과 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, 2와 level 3, 그리고 level 4에서 각각 목표치와 가장 근사한 값을 보였다.

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

REFERENCES