

# Comparison between high-performance liquid chromatography and chemiluminescent immunoassay platforms in determining vitamin D levels in healthy population

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## ABSTRACT

**Background:** Accurate measurement of serum vitamin D level is crucial for diagnosis and managing vitamin D deficiency, as associated with various health problems. High-performance liquid chromatography (HPLC) is commonly used methods for measuring serum vitamin D levels, but limited research has compared their performance with other available and reasonably less expansive method of testing Vitamin D levels like, Chemiluminescent immunoassay (CLIA). This study is intended to see the reliability of CLIA method compared with HPLC in measuring serum 25-hydroxyvitamin D [25(OH)D] levels in apparently healthy individuals.

**Methods:** In this analytical cross-sectional study, we evaluated the reliability of CLIA method and compared to HPLC as standard, in measuring serum 25-hydroxyvitamin D [25(OH)D] levels among apparently healthy individuals. Serum samples from 76 participants were analyzed using both HPLC and CLIA.

**Results:** In the HPLC method, the mean  $\pm$  SD and median of 25(OH)D levels were  $24.73 \pm 17.80$  ng/ml and 19.67 ng/ml, respectively. In the CLIA method, the mean  $\pm$  SD and median of 25(OH)D levels were  $29.96 \pm 21.59$  ng/ml and 22.59 ng/ml, respectively. Our results showed differences in mean and median values of 25(OH) D levels between the two methods, with higher values obtained from CLIA. However, there was a significant correlation between results obtained from both methods, indicating reasonable diagnostic accuracy. The coefficient of variation was higher in CLIA, suggesting higher variability in measurements. The Intraclass Correlation Coefficient for consistency was high in both methods, indicating good agreement between repeated measures. The Area Under the Curve for differentiating normal or low 25(OH) D levels and determining deficiency or not was high for both methods, indicating good diagnostic performance.

**Conclusion:** Our study suggests that while there are differences in results between CLIA and HPLC methods for measuring serum vitamin D levels, both methods show reasonable diagnostic accuracy in a real-world clinical setting. Factors such as laboratory setup, resource availability, and population characteristics should be considered when choosing a method for measuring serum vitamin D levels.

**Key words:** High-performance liquid chromatography (HPLC), Chemiluminescent immunoassay (CLIA), 25(OH)D.

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## INTRODUCTION

Vitamin D is an important nutrient that plays crucial roles in a variety of physiological processes, including bone health, immune function, and the regulation of calcium and phosphorus metabolism. Vitamin D deficiency is described as a pandemic and the deficiency has been linked to a wide range of health problems, such as rickets, osteoporosis, cardiovascular disease, and autoimmune disease.<sup>1-3</sup> Therefore, accurate measurement of serum vitamin D levels is of great clinical importance in the diagnosis and management of vitamin D deficiency.

There are several methods available to measure serum 25(OH)D levels. They can be broadly classified into two categories. One, including physical detection methods as (high performance liquid chromatography [HPLC] and liquid chromatography–tandem mass spectrometry [LC-MS/MS] and other is immunoassays (radio-immunoassay [RIA], enzyme-linked immunosorbent assay [ELISA], chemiluminescent immunoassay [CLIA], lateral flow immunoassay, and assays for clinical chemistry analyzers).

Automated immunoassays are attractive for reasons of high throughput capability and combination with routine chemistries.<sup>4,5</sup> LC-MS has been considered the “Gold standard method” for serum or plasma 25(OH)D measurements but yet not readily available.<sup>6</sup> HPLC is a widely used method that offers high precision and accuracy in measuring vitamin D levels, while CLIA is an immunoassay-based method that is relatively less complex, offers rapid results, and is less expensive.<sup>4,7,8</sup> Both HPLC and CLIA methods have their advantages and limitations, and the choice of method may vary depending on laboratory setup, resource availability, and the specific requirements of the population being studied.

Despite the importance of accurate measurement of serum vitamin D levels, there is limited research comparing the performance of HPLC and CLIA methods. Therefore, our study aimed to evaluate the performance of CLIA methods compared to HPLC methods for measuring serum vitamin D levels in a real-world clinical setting. We measured vitamin D status by analyzing 25-hydroxy vitamin D [25(OH)D] concentration in the blood, as it is much higher than that of active 1,25-dihydroxy vitamin D [1,25-(OH)<sub>2</sub>-vitamin D] and it has a longer half-life of 2-3 weeks, in contrast to 1,25-(OH)<sub>2</sub>-vitamin D, which has a half-life of only 4 hours.<sup>9,10</sup>

## METHODS

We conducted an analytical cross-sectional study to assess the reliability of CLIA methods compared to HPLC methods for measuring serum 25(OH)D levels in apparently healthy individuals.

The study sample included 76 participants, recruited conveniently from people who visited the outpatient department at BIRDEM General Hospital for routine health checkup. Serum samples were collected and analyzed for 25(OH)D levels using both HPLC and CLIA

platforms. CLIA was used to measure by Alinity i analyzer and Alinity i 25-OH Vitamin D Reagent kit 08P45 made by Abbott.

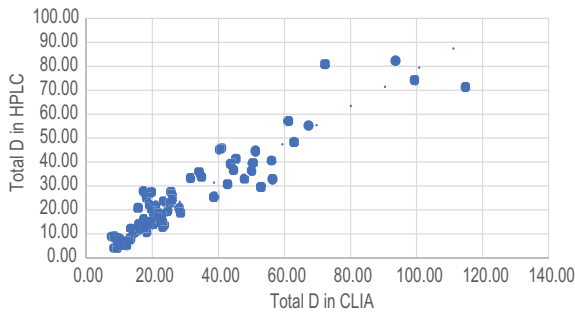
Status of individual patient’s vitamin D intake was not known. Ethical clearance was taken from Ethical Board of BIRDEM Academy. Cost for vitamin D measurement by HPLC method was provided by the patient as a part of routine checkup and test done by CLIA method was funded by the researcher.

## RESULTS

A total of 76 individuals were included in the study and result after analysis showed that 15 (19.7%) being male and 61 (80.3%) being female. In terms of the participants’ profession, the majority of individuals were housewives, comprising 49 individuals (64.5%) of the total sample size. Additionally, there were 12 individuals (15.8%) who reported being engaged in indoor jobs, while 3 individuals (3.9%) reported outdoor jobs. Moreover, 8 individuals (10.5%) identified themselves as students. However, data regarding the profession was unavailable for 4 individuals in the study. The mean age  $\pm$  standard deviation (SD) of the participants was 45.59  $\pm$  15.28 years, and the average body mass index (BMI) was 27.58  $\pm$  4.46 kg/m<sup>2</sup>.

Serum 25(OH)D levels were measured using two different methods named HPLC and CLIA. In the HPLC method, the mean  $\pm$  SD and median of 25(OH)D levels were 24.73  $\pm$  17.80 ng/ml and 19.67 ng/ml, respectively, with a minimum and maximum value of 4.19 ng/ml and 82.26 ng/ml, respectively. In the CLIA method, the mean  $\pm$  SD and median of 25(OH)D levels were 29.96  $\pm$  21.59 ng/ml and 22.59 ng/ml, respectively, with a minimum and maximum value of 7.59 ng/ml and 114.75 ng/ml, respectively. Based on the Endocrine Society definition in HPLC method 22(28.9%) individuals had normal vitamin D levels, 15(19.7%) had vitamin D insufficiency and 39(51%) had vitamin D deficiency, whereas in CLIA methods 24(31.6%) individuals had normal vitamin D level, 20(26.3%) had vitamin D insufficiency and 32(42.1%) had vitamin D deficiency.<sup>11</sup>

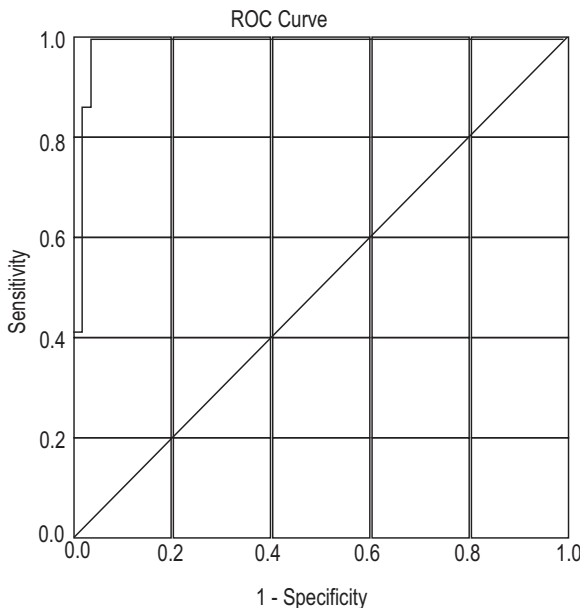
The coefficient of variation (CV) was 316.74 in the HPLC method and 464.97 in the CLIA method. The Spearman’s correlation coefficient for the 25(OH)D levels was found to be 0.911, with a statistically significant p-value of 0.000 (Figure 1).



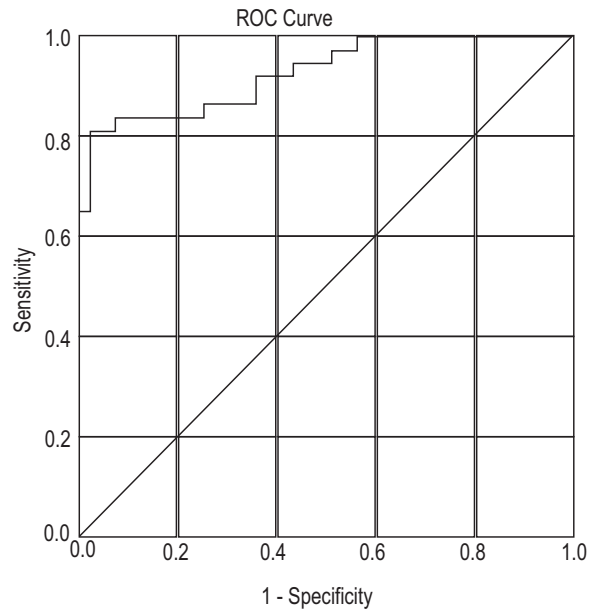
**Figure 1.** The Spearman correlation coefficient of the 25(OH)D between HPLC and CLIA methods

The Intraclass Correlation Coefficient (ICC) for consistency was 0.918 (95% CI: 0.873, 0.947) in single measures and 0.957 (95% CI: 0.932, 0.973) in average measures.

The Area Under the Curve (AUC) for differentiating normal or low 25(OH)D levels by comparing CLIA method to HPLC method was 0.987 (p-value: 0.000; 95% CI: 0.964, 1.00) (Figure 2). The AUC for determining deficient or not was 0.927 (p-value: 0.000; 95% CI: 0.869, 0.984) (Figure 3).



**Figure 2.** ROC curve for differentiating normal or low 25(OH)D levels by comparing CLIA method to HPLC method.



**Figure 3.** ROC curve for differentiating deficient or not by comparing CLIA method to HPLC method.

**DISCUSSION**

Our study was aimed to compare the reliability of vitamin D assay between HPLC and CLIA methods. The results of our study revealed some differences in the results obtained from these two methods when measuring serum 25(OH)D levels in apparently healthy individuals. However, despite these differences, there was a significant correlation between the results obtained from both methods, indicating a reasonable diagnostic accuracy between the two methods.

In our study, we found that the mean and median values of 25(OH)D levels were higher when measured by CLIA compared to HPLC. Additionally, the proportion of individuals categorized as having normal vitamin D levels, vitamin D insufficiency, and vitamin D deficiency differed between the two methods, with CLIA method showing higher proportions of individuals with normal vitamin D levels and vitamin D insufficiency compared to HPLC method. These differences in results could be attributed to the inherent differences in the principles and techniques of HPLC and CLIA methods.

The differences in the results obtained from HPLC and CLIA methods in our study are consistent with previous studies that have reported discrepancies between these two methods.<sup>12</sup> The coefficient of variation (CV) in our study was found to be higher in

the CLIA method compared to the HPLC method, indicating that the CLIA method may have higher variability and less precision compared to HPLC. However, despite this difference, the Spearman's correlation coefficient between the two methods was high, indicating a strong correlation between the results obtained from HPLC and CLIA.

Moreover, the Intraclass Correlation Coefficient (ICC) for consistency, both in the single ICC and average ICC values, was found to be above 0.90. This suggests a high level of agreement or consistency among the measurements being compared. Additionally, if the average ICC value is higher than the single ICC value, it indicates that the overall level of agreement or consistency is even higher when considering the average values, compared to individual measurements. This suggests that the measurements obtained from both methods are highly reliable and consistent with each other, as both the individual and average ICC values are above the commonly used threshold of 0.90, indicating excellent agreement.

The AUC for differentiating normal or low 25(OH)D levels and determining deficient or not was high, indicating the good discriminatory ability of CLIA compared to HPLC in our study. However, it is important to note that the AUC value may not be the only factor to consider when evaluating the performance of diagnostic tests, as it depends on the prevalence of the condition, the clinical context, and the specific cutoff values used.<sup>13</sup>

These findings suggest that while there are differences in the absolute values of 25(OH)D levels obtained from the HPLC and CLIA methods, there is good consistency and agreement between the two methods in categorizing individuals into different vitamin D status categories. The CLIA method may be a reliable alternative to the HPLC method for measuring serum 25(OH)D levels in a clinical setting, considering its relatively less complexity, rapid results, good accuracy and less expense.

However, further studies with larger sample sizes and diverse populations are needed to confirm our findings and to assess the clinical implications of these differences in vitamin D assay methods and can provide

more robust evidence on the performance of HPLC and CLIA methods in measuring vitamin D levels.

### Conclusion

Our study suggests that CLIA could be used with confidence to measure vitamin D levels when HPLC methods are not available or not affordable due to higher costs.

**Authors' contribution:** AM and WMMH were involved in sample collection and manuscript writing. WMMH performed test and data analysis. MFP reviewed manuscript and data analysis.

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**Conflicts of interest:** Nothing to declare

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