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(RESEARCH ARTICLE)

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Verification of analytical performance of the 25-OH vitamin D assay on the Abbott Alinity ci®: Experience of the central laboratory of Mohammed VI University Hospital of Oujda

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Abstract

The primary goal of our study was to verify the analytical performance of the 25-OH Vitamin D measurements conducted on the Abbott CI analyzer using the immuno-chemiluminescence method. This verification took place in the biochemistry laboratory at Mohammed VI University Hospital in Oujda.

Our methodology was aligned with the recommendations outlined in the French accreditation committee (COFRAC) technical guide (GTA) 04, emphasizing the assessment of reproducibility and repeatability.

Overall, the results of this evaluation were satisfactory and met the standards set by both the supplier and the French Society of Clinical Biology. This study illustrates that the biochemistry laboratory at Mohammed VI University Hospital of Oujda is equipped to provide accurate and precise results, which are crucial for effective clinical diagnosis and decision-making.

Keywords: 25-OH Vitamin D assay; Analytical performance; Repeatability; Reproducibility; Alinity CI analyzer; Immuno-chemiluminescence

1. Introduction

Ensuring high standards in laboratory testing requires a steadfast dedication to obtaining precise results at all times. A robust quality assurance framework includes every aspect of laboratory work, both internal and external, while incorporating best practices and enhanced management techniques. This structure guarantees that tests are performed correctly on suitable samples from the right individuals in properly equipped environments, resulting in trustworthy interpretations grounded in reliable reference data. To instill quality principles in medical laboratories, it is crucial to develop a focused quality management program that maintains the integrity of the results produced.

In recent years, significant advancements have been made to enhance quality in clinical laboratories, particularly through the implementation of accreditation based on ISO 15189 standards, which assess both technical and managerial capabilities. This accreditation process includes the validation, verification, and quality assurance of testing methods.

Accredited laboratories are required to evaluate and document the analytical performance of all methods not only prior to their implementation but also throughout their operational lifecycle. Clear, standardized, and practical guidelines are critical in this regard [1, 2].

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The verification of analytical methods in medical laboratories is vital to ensure that results accurately reflect the true reference values of samples, thereby guaranteeing the accuracy and reliability of measurements. This process involves a series of steps designed to meet the quality standards outlined in ISO 15189, including assessing the effectiveness of analytical procedures, measuring performance through standardized approaches, and comparing results against predefined benchmarks [3].

Improper implementation of these methods can lead to inaccurate assessments of method performance, potentially compromising patient safety and leading to incorrect diagnoses.

The central laboratory at Mohammed VI University Hospital in Oujda has developed a comprehensive quality strategy that includes a method verification protocol, which is integral to our study. Our research will focus on evaluating the analytical performance of the 25-OH Vitamin D measurements, using the criteria specified in Scope A of the detailed medical biology method verification and validation guide.

1.1. Interest of 25-OH Vitamin D determination

Vitamin D is a fat-soluble steroid prohormone mainly produced photochemically in the skin from 7-dehydrocholesterol. Two forms of vitamin D are biologically relevant - vitamin D3 (Cholecalciferol) and vitamin D2 (Ergocalciferol). Both vitamins D3 and D2 can be absorbed from food, with vitamin D2 being an artificial source, but only an estimated 10-20% of vitamin D is supplied through nutritional intake. Vitamins D3 and D2 can be found in vitamin supplements. Vitamin D is converted to the active hormone 1,25-(OH)2-vitamin D (Calcitriol) through two hydroxylation reactions. The first hydroxylation converts vitamin D into 25-OH vitamin D and occurs in the liver. The second hydroxylation converts 25-OH vitamin D into the biologically active 1,25-(OH)2-vitamin D and occurs in the kidneys as well as in many other cells of the body. Most cells express the vitamin D receptor and about 3% of the human genome is directly or indirectly regulated by the vitamin D endocrine system.

The major storage form of vitamin D is 25-OH vitamin D and is present in the blood at up to 1000 fold higher concentration compared to the active 1,25-(OH)2-vitamin D. 25-OH vitamin D has a half-life of 2-3 weeks vs. 4 hours for 1,25-(OH)2-vitamin D. Therefore, 25-OH vitamin D is the analyte of choice for determination of the vitamin D status.

Epidemiological studies have shown a high global prevalence of vitamin D insufficiency and deficiency. Risk factors for vitamin D deficiency include low sun exposure, malnutrition, some malabsorption syndromes, and liver or kidney diseases.10 The measurement of vitamin D status provides opportunities for preventive and therapeutic interventions.

Vitamin D deficiency is a cause of secondary hyperparathyroidism and diseases resulting in impaired bone metabolism (like rickets, osteoporosis, osteomalacia) [4, 5].

1.2. Principle of 25-OH Vitamin D assay method

This assay is a delayed one-step immunoassay for the quantitative determination of 25-OH vitamin D in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA) technology.

Sample, anti-vitamin D coated paramagnetic microparticles, and assay diluent are combined and incubated. The 25-OH vitamin D present in the sample is displaced from the vitamin D binding protein and binds to the anti-vitamin D coated microparticles. Vitamin D acridinium-labeled conjugate is added to create a reaction mixture. The reaction mixture is incubated. Following a wash cycle, Pre- Trigger and Trigger Solutions are added.

The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is a relationship between the amount of 25-OH vitamin D in the sample and the RLUS detected by the system optics [6]

2. Material and methods

This prospective study was conducted over a 30-day period in the biochemistry laboratory at Mohammed VI University Hospital.

Our research consisted of two phases. The first phase focused on evaluating reproducibility, also known as intermediate fidelity, by performing daily internal controls across three measurement levels: low, medium, and high. This was done to assess consistency over the 30 days. For this phase, we selected a range of serum samples with 25-OH Vitamin D

values that were evenly distributed across the measurement spectrum. These samples were categorized into three groups: low, medium, and high based on their 25-OH Vitamin D levels.

In the second phase, we assessed repeatability by running thirty repetitions of each sample. The analytical procedure utilized the Alinity i® 25-OH Vitamin D reagent kit on the immunoassay system.

Our operational approach was guided by the recommendations outlined in the COFRAC GTA 04 accreditation technical guide. Statistical analysis of the data was performed using the EVM intermediate module from BYG Informatics.

3. Results

3.1. Reproducibility results

Intra-laboratory reproducibility, also known as intermediate fidelity, is assessed by repeatedly measuring samples under various operational conditions (such as time, reagent batches, calibrations, operators, and equipment) to evaluate how these factors affect the results.

The collected data is used to calculate the mean, standard deviation, and coefficient of variation (CV) for each series, as well as within-series and between-series comparisons, and for the overall data set [7].

The intermediate fidelity outcomes were acceptable across the three levels: low, medium and high, with coefficients of variation (CV) of 3.28%, 3.71% and 3.76% respectively. By comparing these results with the CV retained by manufacturer's specifications, we note that the results are in conformity and inferior to the tolerated limits.

To visually represent these results, Levey-Jennings plots (Fig. 1, Fig. 2, and Fig. 3) have been created, enhancing the clarity of the findings.

Table 1 Reproducibility results of blood assay by level with comparison to manufacturer's specifications

Level of IQC	Number of values	Mean (g/l)	Standard Deviation	Coefficient of Variation CV (%)	CV Manufacturer's specifications (%)
Low	30	17.59 ng/ml	0.578 ng/ml	3.28 %	3.30 %
Medium	30	40.01 ng/ml	1.484 ng/ml	3.71 %	4.60 %
High	30	75.04 ng/ml	2.821 ng/ml	3.76 %	3.80 %

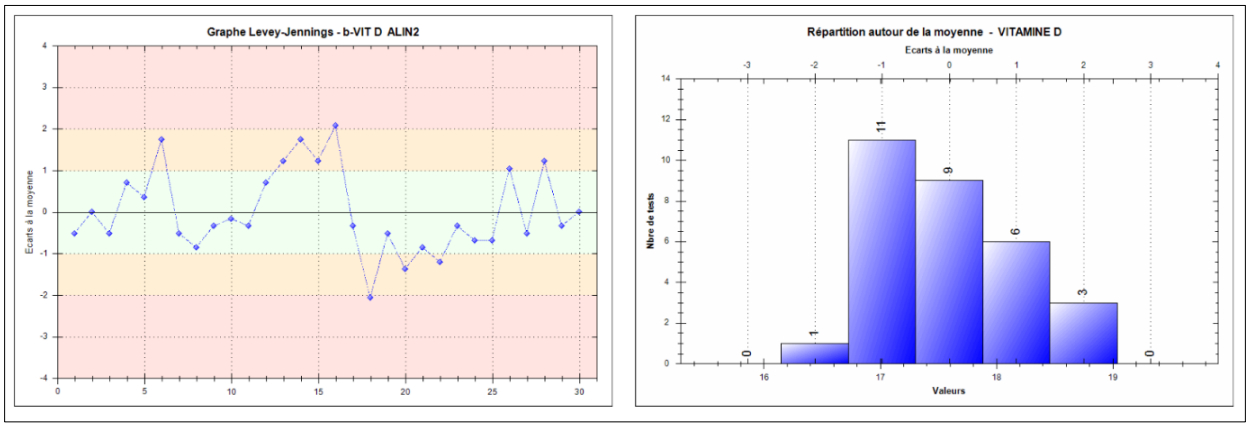

Figure 1 Low Level of Reproducibility: Levey Jennings graph and the distribution around the mean - 25-OH Vitamin D

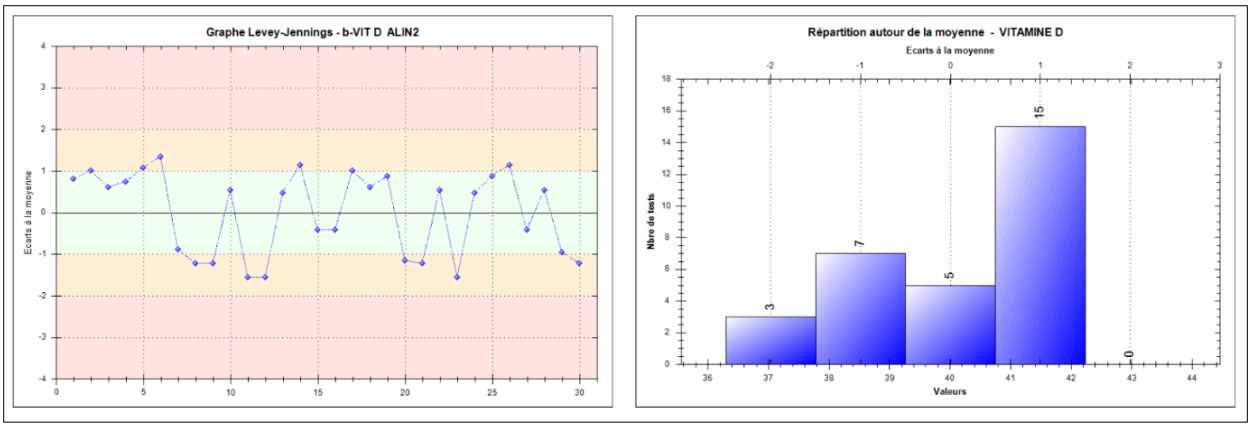

Figure 2 Medium Level of Reproducibility: Levey Jennings graph and the distribution around the mean - 25-OH Vitamin D

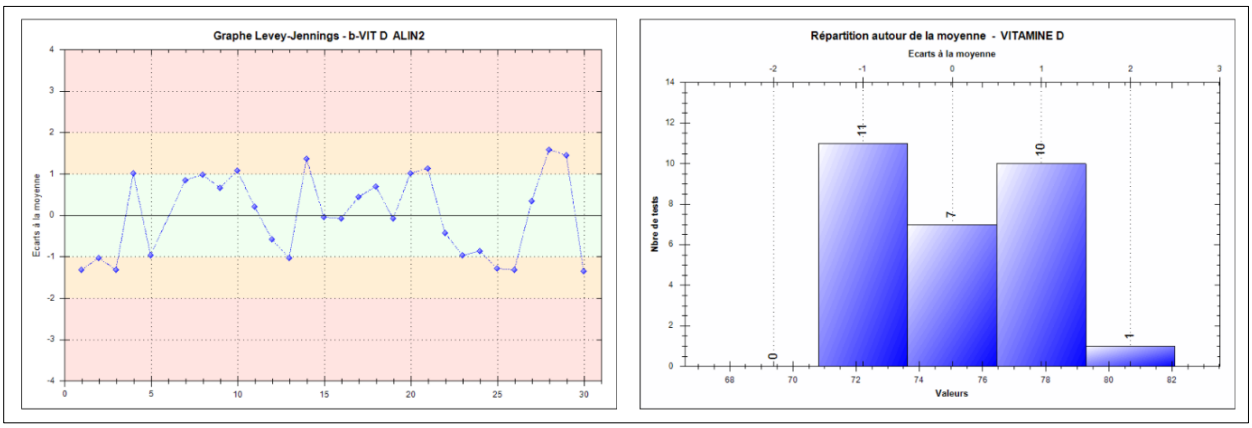

Figure 3 High Level of Reproducibility: Levey Jennings graph and the distribution around the mean - 25-OH Vitamin D

3.2. Repeatability Results

Repeatability is evaluated by conducting multiple assays of the same samples by the same operator under consistent conditions, which include factors such as reagent, calibration, instrument, and operator, all within the shortest possible timeframe.

This repeatability assessment allows for the determination of initial performance and verifies the proper functioning of the system (instrument and reagent) for the specific analyte. Variability is again measured using coefficient of variation (CV) values [7].

As shown in Table 2, the results for the various verification criteria of the 25-OH Vitamin D assay indicate satisfactory repeatability across all three levels: low, medium, and high, with coefficients of variation of 2.71%, 2.16%, and 2.52%, respectively, based on 30 samples.

Table 2 Repeatability results for 25-OH Vitamin D on the Alinity i® automated system by level with comparison to manufacturer's specifications

Level of IQC	Number of values	Mean (g/l)	Standard Deviation	Coefficient of Variation CV (%)	CV Manufacturer's specifications (%)
Low	30	20.62 ng/ml	0.560 ng/ml	2.71 %	2.80 %
Medium	30	40.57 ng/ml	0.876 ng/ml	2.16 %	3.60 %
High	30	76.74 ng/ml	1.930 ng/ml	2.52 %	3.30 %

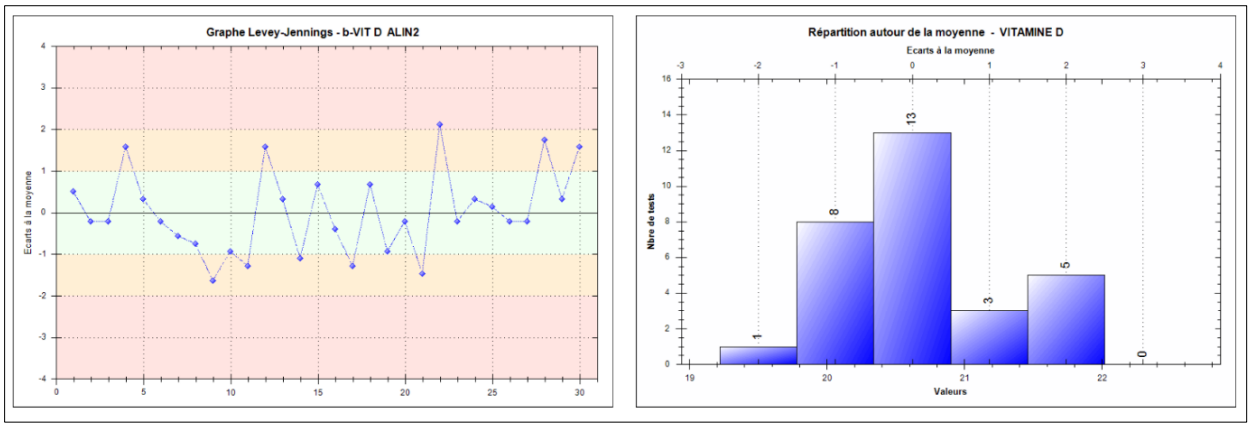

Figure 4 Low Level of Repeatability: Levey Jennings graph and the distribution around the mean - 25-OH Vitamin D

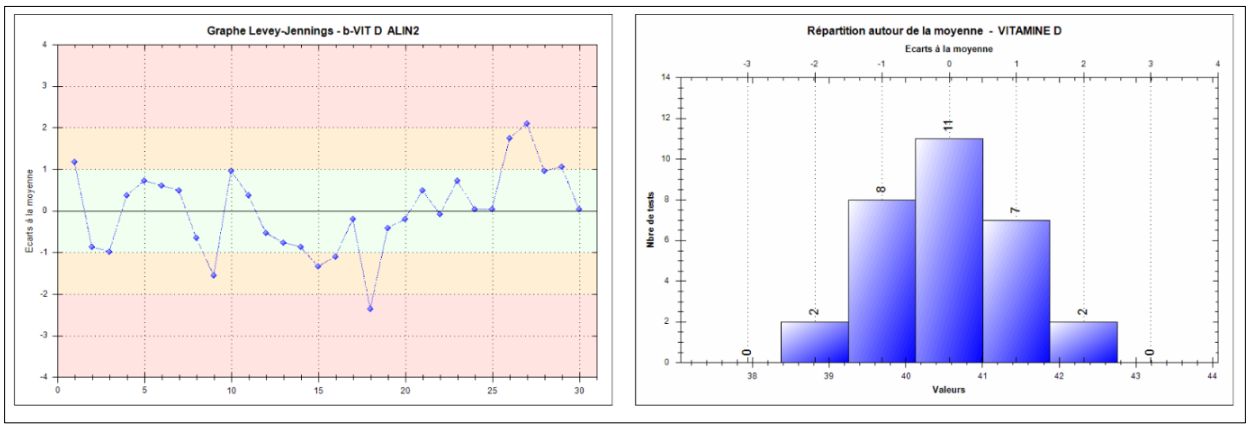

Figure 5 Medium Level of Repeatability: Levey Jennings graph and the distribution around the mean - 25-OH Vitamin D

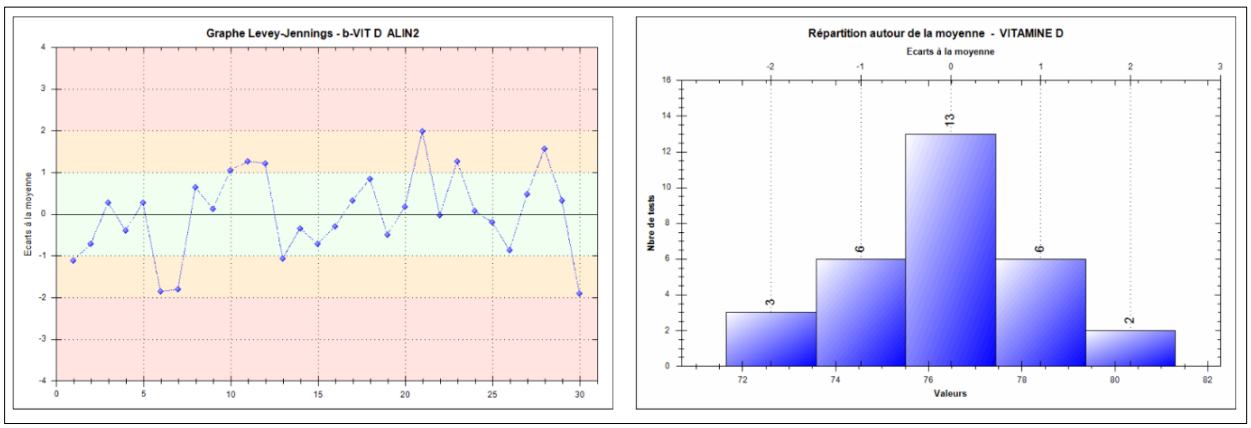

Figure 6 High Level of Repeatability: Levey Jennings graph and the distribution around the mean - 25-OH Vitamin D

4. Discussion

Beyond its well-known effects on calcium-phosphate metabolism, vitamin D has numerous effects that have been the subject of a multitude of publications in recent years. Severe vitamin D deficiency is responsible for osteomalacia in adults and rickets in children. However, before discussing a true deficiency in vitamin D, there is an intermediate stage known as "vitamin D insufficiency," the main consequence of which, particularly in the elderly, is the development of

secondary hyperparathyroidism, leading to an increased risk of fractures. The increase in parathyroid hormone (PTH) concentrations, however, does not always exceed physiological limits. Therefore, it is important to detect these "insufficiencies" in vitamin D, as supplementation with vitamin D and calcium can decrease PTH levels and bone resorption, significantly reducing the incidence of non-vertebral fractures.

The Abbott Alinity ci® system is a comprehensive multiparametric platform that integrates clinical chemistry with immunoassay capabilities, facilitating the measurement of a wide array of standard biochemical parameters and specific proteins. For the 25-OH Vitamin D assay, the CMIA (microparticle chemiluminescence immunoassay) method is employed, which eliminates the need for validation; instead, we focus on verification following the “scope A verification/validation” framework. This ensures that recognized methods are pre-validated within their intended applications, thus confirming the accuracy and reliability of the results obtained [8].

This verification process is essential for adherence to regulatory standards outlined in the Moroccan Guide for the Proper Execution of Medical Laboratory Analyses (GBEA) and complies with ISO 15189:2022 requirements. By setting clear analytical goals through this control, we can ensure the generation of accurate and trustworthy results.

A reproducibility test is conducted to evaluate the consistency of assay results when different variables are introduced. Our study confirmed the reliability of the 25-OH Vitamin D assay for reproducibility assessment across three distinct levels: low, medium, and high. Each level involved the analysis of 30 values, resulting in means of m1 = 17.59 ng/ml, m2 = 40.01 ng/ml, and m3 = 75.04 ng/ml, with coefficients of variation (CV) of CV1 = 3.28%, CV2 = 3.71%, and CV3 = 3.76%. The low CV values indicate consistent results that remain close to the mean, even when various factors are adjusted. This reliability is crucial in medical testing, where consistent results are vital for clinical decision-making. The fact that the CV values align with manufacturer’s specifications indicates compliance with industry standards for reproducibility, thereby enhancing its suitability for accurate diagnostics.

The focus of the repeatability test is on the precision of the assay under controlled conditions. This evaluation is important as it measures the method’s capability to yield consistent results when the same sample is analyzed repeatedly. In our assessment of repeatability at three levels (low, medium, and high), we analyzed 30 values for each, resulting in remarkably low coefficients of variation (CV): CV1 = 2.71%, CV2 = 2.16%, and CV3 = 2.52%. These low CV values reflect minimal variability, underscoring the assay’s high precision.

The stability and predictability of the assay's outcomes under controlled conditions are critical, especially in clinical settings where minor variations can significantly affect patient care.

At the Mohammed VI University Hospital in Oujda, our laboratory has implemented a comprehensive quality strategy that includes a robust method verification protocol. This investigation is vital for establishing a credible accreditation pathway for the analyses conducted. Serving as a primary reference center in Eastern Morocco, our laboratory not only addresses the needs of referred and hospitalized patients but also significantly contributes to understanding the overall health of the regional population through various scientific initiatives [9, 10].

5. Conclusion

The role of medical biology has become essential in the healthcare landscape, evolving the choice of analytical methods from random selections to a systematic approach driven by defined criteria based on the principles of the technique and its validation or verification processes. At the Mohammed VI University Hospital's central laboratory, there is a strong commitment to the accreditation process, with method validation and verification recognized as vital steps in this endeavor. The results for reproducibility and repeatability are exceptional, conforming to the manufacturer’s specifications. These results strongly indicate the robustness and reliability of the serum 25-OH Vitamin D assay. This investigation underscores the rigorous quality control practices in medical laboratories, enriching the essential knowledge base necessary for accurate serum 25-OH Vitamin D measurements and thereby improving the clinical relevance of this assay.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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