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# Verification of the analytical performance of the serum CA 125 assay on the Abbott Alinity ci®: Experience of the biochemistry laboratory of the Mohammed VI university hospital of Oujda

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## Abstract

The aim of our work was to evaluate the analytical performance of CA 125 determination by a two-step immunoassay using microparticle chemiluminescence immunoassay (CMIA) technology, in accordance with the Scope A criteria of the guide of the verification/validation of medical biology methods.

We evaluated the repeatability and the intermediate precision of the CA 125 assay.The results obtained are very satisfactory for the three levels (low, medium and high), both for intermediate fidelity, with coefficients of variation (CV) of 2.68%, 1.62% and 2.11% respectively, and for repeatability, with coefficients of variation of CV1 = 2.17%, CV2 = 2.04%, and CV3 = 1.38% respectively.

The results obtained made it possible to verify the method's performance and compare it with the analytical objectives set in order to meet the regulatory and normative requirements set by the supplier and learned societies.

The achieved results facilitated the verification of the method's performance and its comparison with the analytical objectives established, aligning with regulatory and normative requirements outlined by the supplier and relevant professional learned societies.

**Keywords:** CA125; Verification; Repeatability; Reproducibility; Alinity ci

## 1. Introduction

Quality is a continuous process focused on achieving accurate test performance consistently, without fail. A quality assurance system encompasses all internal and external laboratory activities, integrating proper laboratory practices and improved management skills to guarantee that tests are conducted accurately on appropriate samples obtained from the correct subjects, at well-equipped facilities. This results in precise interpretation based on accurate reference data. The incorporation of quality principles in medical laboratories necessitates the establishment of a targeted quality management program to uphold the reliability of laboratory-generated results.

Major efforts have been made in recent years to improve quality within clinical laboratories, notably through the implementation of accreditation according to ISO 15189 standards, which covers both technical and management capabilities of a laboratory. The accreditation process involves validation, verification, and quality assurance of methods.

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Indeed, accredited laboratories are required to assess and document the analytical performance of all methods, not only before their initial implementation but also during their ongoing operation. Clear, standardized, and practical guidelines in this area are necessary (1),(2).

The verification of an analytical method within a medical laboratory is vital to guarantee that the outcome closely aligns with the genuine reference value of a sample, ensuring accuracy and dependability in measurements. This process involves a series of actions conducted to fulfill the quality standards outlined in the ISO 15189 standard. Essentially, it encompasses assessing the effectiveness of the analytical procedure, measuring its performance through a standardized operational approach, and subsequently assessing it against predefined benchmarks (3).

An incorrect implementation of these methods can lead to erroneous conclusions regarding method performance, potentially compromising patient safety or contributing to incorrect diagnoses.

The central laboratory of the Mohammed VI University Hospital in Oujda has instituted a quality strategy encompassing a method verification protocol, of which our study is an integral component.

Our work will assess the analytical performance of the measurement of a commonly used tumor marker, CA 125, using the criteria outlined in Scope A of the detailed description provided in the medical biology method verification/validation guide.

### 1.1. Reminder on CA 125

CA125 (also known as MUC16), encoded by the homonymous MUC16 gene, is a giant high molecular weight transmembrane glycoprotein that is highly glycosylated .It is composed of three main parts: the N-terminal domain, the tandem repeat domain with repeating sequences that are rich in serine, threonine and proline and the C-terminal domain which contains multiple extracellular SEA modules (sea urchin sperm protein, enterokinase, and agrin), along with a transmembrane domain (TM) and a brief cytoplasmic tail (CT) of 31 amino acids (4) (5).

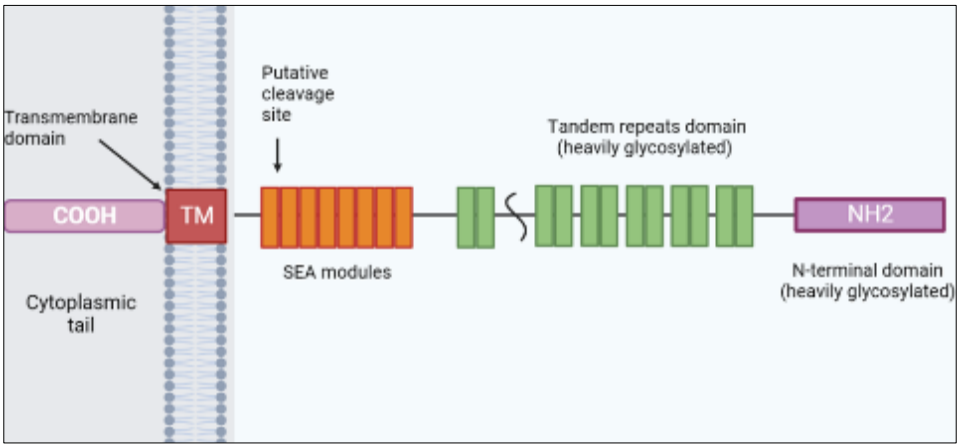


**Figure 1** Structure of CA 125 (5)

Studies reveal that CA125 originates from the cell surfaces of various tissues of the coelomic epithelium. Additionally, it's expressed by the mesothelial cells of the peritoneum and all other Mullerian tissues, including the tubal endothelium, the endocervix, and others.Therefore, CA 125 is mainly associated with epithelial tumours, in particular epithelial ovarian cancer (6).

Its key function is to lubricate, hydrate, and protect the epithelial cavity's surface from physical pressure, infections and injuries (4).

In clinical practice, the CA 125 assay is of great interest as a biomarker for predicting the prognosis of ovarian cancer and for monitoring the response to treatment in patients with epithelial ovarian cancer.

The use of CA 125 ,alone ,as a tumour biomarker for screening and diagnosis of ovarian cancer is limited due to its poor specificity and sensitivity, particularly in the precocious stages of the disease, which can frequently lead to false negatives with important clinical implications (4).

Numerous research studies also indicate that CA125 shows high sensitivity to tissue congestion, presenting potential for effectively monitoring and optimally treating congestive heart failure (HF). Moreover, in assessing right heart function parameters, CA125 levels prove more beneficial compared to other heart failure biomarkers. CA125 is anticipated to emerge as a novel biological marker for congestive HF, and as a result, is expected to see widespread adoption in clinical practice (5).

### 1.2. Principle of the assay method

CA 125 was identified by Bast et al. in 1981 through the creation of a murine monoclonal antibody (OC 125). This antibody was developed by immunizing a mouse with the OVCA 433 cell line, originating from a patient with ovarian serous carcinoma. The initial immunoassay for CA 125, introduced and marketed , used the OC 125 antibody for both capture and detection (7).

Alinity i CA 125 II is a second generation assay for the quantitative determination of antigen in human serum and plasma using the monoclonal antibody OC 125 as the capture antibody.

The assay is a two-step immunoassay using microparticle chemiluminescence immunoassay (CMIA) technology.

The sample and paramagnetic microparticles coated with OC 125 antibody are brought together and incubated. The OC 125 antibody-defined antigen present in the sample binds to the OC 125 antibody-coated microparticles.

These defined antigens are quantified using acridinium-labelled M11 antibodies.

The resulting chemiluminescent reaction, after addition of the pre-activation and activation solutions, is measured in relative light units (RLU).

There is a direct relationship between the amount of antigen defined by the OC 125 antibody present in the sample and the relative light units (RLU) detected by the optical system.

## 2. Material and methods

This is a prospective study that was carried out over a 30 days period in the biochemistry laboratory of the Mohammed VI University Hospital.

Our study involved two phases; the first phase aimed to evaluate the reproducibility also called intermediate fidelity by daily running internal controls on the three measurement levels : low, medium, and high ; over a period of 30 days, to evaluate the consistency.

In this first phase we selected a set of serum samples with CA 125 values evenly distributed over the measurement spectrum. The CA 125 levels of these samples were then used to divide them into three groups: low, medium, and high. To evaluate repeatability, thirty repetitions of each sample were run in the second phase.

The analytical procedure was executed employing the Alinity i CA 125 II reagent kit on the immunoassay system.

We implemented an operational approach guided by the recommendations of the COFRAC GTA 04 accreditation technical guide protocol.

The statistical analysis of the data was conducted utilizing the EVM intermediate module provided by BYG Informatics.

## 3. Results

### 3.1. Reproducibility results

Intra-laboratory reproducibility or (Intermediate fidelity) is determined by the repeated measurement of samples under varying operating conditions (time, batches of reagents, calibrations, operators and equipment) to assess the impact of these factors on results.

The data is used to calculate the mean, standard deviation and CV (%) for each series, within series, between series and for all data (8).

The intermediate fidelity outcomes were acceptable across the three levels—low, medium, and high, with coefficients of variation (CV) of 2.68%, 1.62%, and 2.11% respectively. The reproducibility CV for each tier is satisfactory, remaining below the established limits set by both the SFBC (quality control system) and the RICOS (global quality control network).

The results have been graphically depicted through Levey-Jennings plots (Fig. 2, Fig. 3, and Fig. 4) to enhance the clarity of the findings.

**Table 1** Reproducibility results of blood assay by level with comparison to FSBC and RICOS data

| Level of IQC | Number of values | Mean ( g/l) | Standard Deviation | Coefficient of Variation CV (%) | CV SFBC 1999 (%) | CV RICOS S. (2014) (%) |
|---|---|---|---|---|---|---|
| Low | 30 | 19.15 UI/ml | 0.512 UI/ml | 2.68 % | 9.00 % | 12.35 % |
| Medium | 30 | 46.56 UI/ml | 0.754 UI/ml | 1.62 % | 7.00 % | 12.35 % |
| High | 30 | 77.35 UI/ml | 1.634 UI/ml | 2.11 % | 7.00 % | 12.35 % |

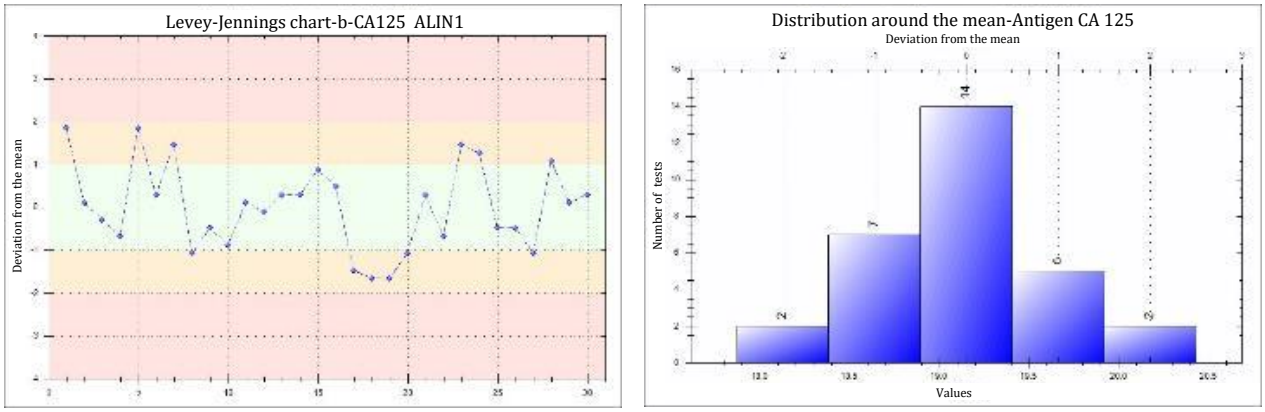


**Figure 2** Low Level of Reproducibility: Levey Jennings graph and the distribution around the mean – CA 125

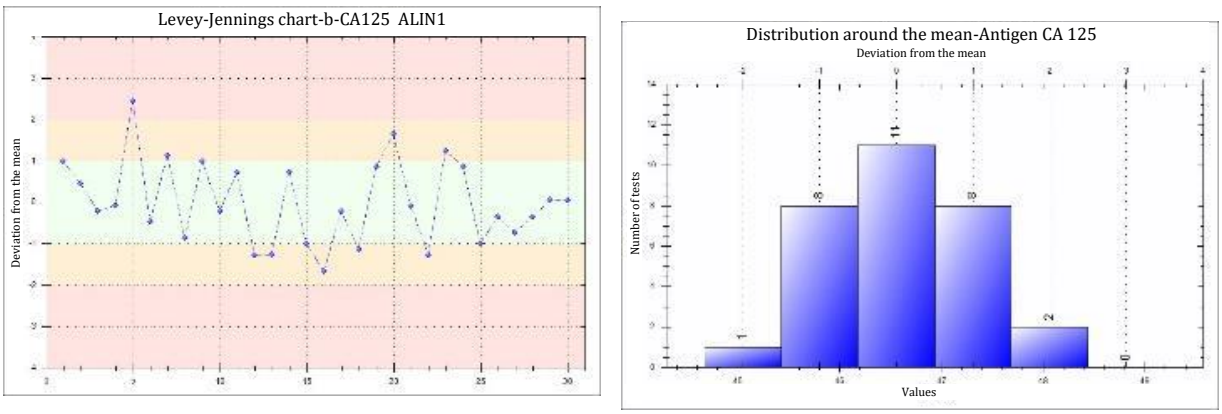


**Figure 3** Medium Level of Reproducibility: Levey Jennings graph and the distribution around the mean – CA 125

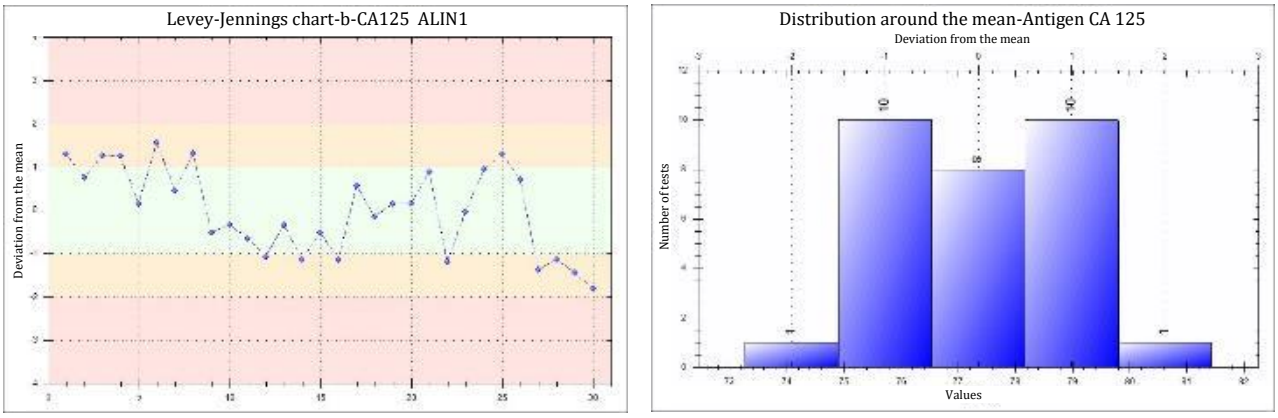


**Figure 4** High Level of Reproducibility: Levey Jennings graph and the distribution around the mean – CA 125

### 3.2. Repeatability Results

Repeatability is assessed through the repeated assay of the same samples by the same operator under uniform conditions, encompassing all aspects of the measurements such as reagent, calibration, instrument, and operator and in the briefest time frame possible.

The repeatability test enables the initial performance to be determined and the correct operation of the system (instrument/reagent) to be verified for the analyte concerned (8).

Once more, variability is measured using CV values.

As indicated in Table 2, the results obtained for the various CA125 assay verification criteria demonstrate satisfactory repetability for all three levels :low, medium, and high, with coefficients of variation (CV) of 2,17 %, 2,04%, and 1,38% respectively on 30 samples.

**Table 2** Repeatability results for CA 125 on the Alinity i® automated system by level with comparison to SFBC and RICOS data

| Level of IQC | Number of values | Mean ( UI/ml) | Standard Deviation ( UI/ml) | Coefficient of Variation CV (%) | CV SFBC 1999 (%) | CV RICOS S. (2014) (%) |
|---|---|---|---|---|---|---|
| Low | 30 | 21,04 | 0,456 | 2,17% | 6,75% | 9,26% |
| Medium | 30 | 23,85 | 0,487 | 2,04% | 5,25% | 9,26% |
| High | 30 | 71,03 | 0,983 | 1,38% | 5,25% | 9,26% |

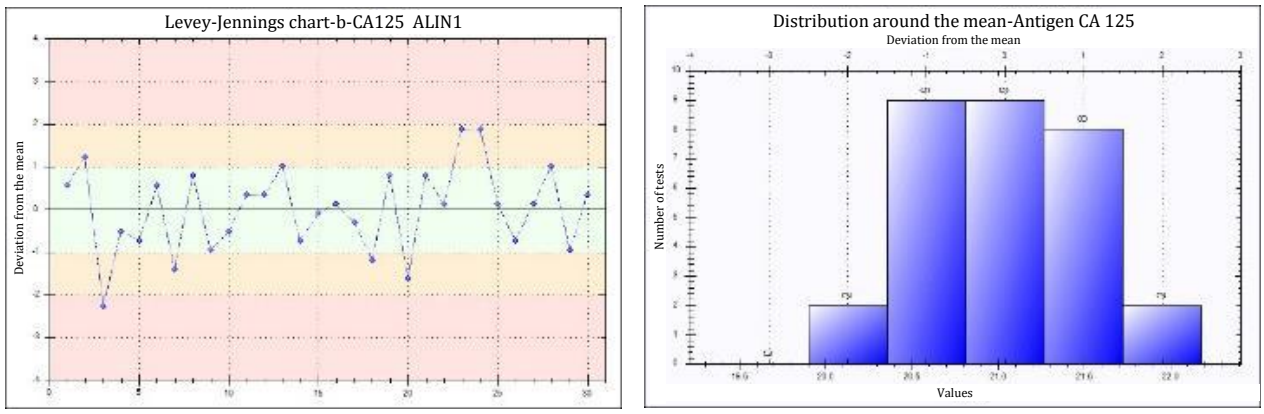


**Figure 5** Low Level of Repeatability: Levey Jennings graph and the distribution around the mean – CA 125

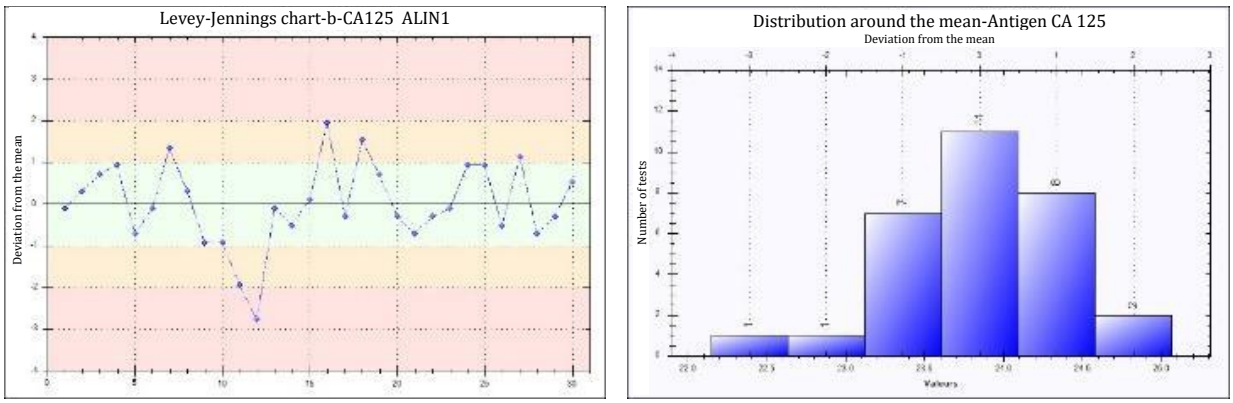


**Figure 6** Medium Level of Repeatability: Levey Jennings graph and the distribution around the mean – CA 125

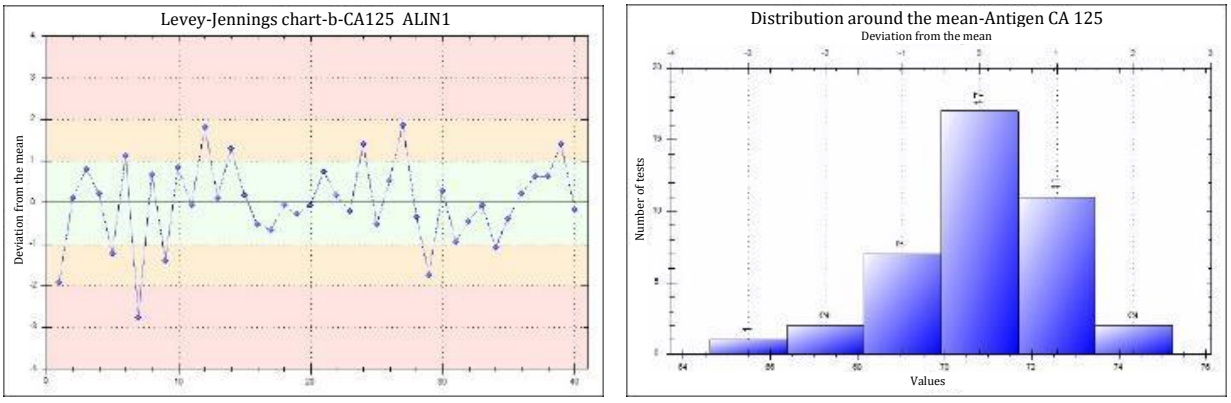


**Figure 7** High Level of repeatability : Levey Jennings graph and the distribution around the mean – CA 125

## 4. Discussion

Ovarian cancer ranks as the seventh most prevalent cancer among women globally (18th overall) and is the second most frequent gynecological cancer following uterine cancer (4).

CA 125 is a widely used tumor marker for diagnosis and monitoring of ovarian cancer.However, relying solely on CA 125 for diagnosis is not sufficient for effective screening and differential diagnosis of ovarian cancer due to its poor sensitivity and specificity.

This limitation arises from the fact that CA 125 does not show increased levels in certain histological types of ovarian cancer. Moreover, there is a notable occurrence of false positives in benign gynecological conditions like ovarian cysts and uterine myomas. Elevated CA125 levels can also be detected in diverse physiological or pathological situations, including early pregnancy, menstruation, peritoneal injury, and ascites stemming from any cause.

A single measurement of CA 125 cannot be interpreted, without the incorporation of other diagnostic techniques, as conclusive evidence of the presence or absence of disease (5),(9).

On the other hand, many consider CA 125 measurement to be standard practice for monitoring ovarian cancer patients and evaluating their prognosis. The principal FDA-approved use of CA 125 in individuals with epithelial ovarian cancer involves monitoring their response to therapeutic treatment (4).

CA125 is also expected to emerge as a new alternative biomarker for congestive heart failure, making it imperative for widespread adoption in clinical settings. Numerous studies have underscored the significance of CA125 as a biomarker that mirrors congestion in connection with the severity of the disease, hemodynamics, and echocardiographic parameters (5).

Effectively utilizing CA125, especially for monitoring and prognostic assessment, necessitates the biologist in the laboratory to master the employed method and the verification/validation of the process.

The Abbott Alinity ci is a multiparametric system capable of integrating clinical chemistry and immunoassay, enabling the measurement of a wide range of standard biochemical parameters as well as specific proteins.

The CMIA (microparticle chemiluminescence immunoassay) method is already being utilized for the CA 125 assay. As a result, validation is not necessary; instead, we only need to conduct verification according to a ‘‘scope A verification/validation” where the recognized methods, are pre-validated within their designated field of application, to ensure the accuracy and the reliability of our results (10).

This verification is essential, meeting both regulatory standards (as per the Moroccan Guide for the Proper Execution of Medical Laboratory Analyses GBEA) and normative requirements (ISO 15189 :2022). Setting predetermined analytical goals through this control ensures the production of precise and dependable results.

The reproducibility test is employed to assess the consistency of assay results when different variables are introduced. Our study results affirmed the reliability of the CA 125 assay for reproducibility assessment. The three levels—low, medium, and high—yielded satisfactory outcomes. For each level, 30 values were analyzed, revealing means of m1 = 19.15 IU/ml, m2 = 46.56 IU/ml, and m3 = 77.35 IU/ml, along with coefficients of variation (CV) of CV1 = 2.68%, CV2 = 1.62%, and CV3 = 2.11%.

The low CV values signify that even when modifying various factors, the test consistently produces results close to the mean value. This reliability is crucial in medical testing, where consistency ensures the dependability of test results for clinical decisions. The fact that CV values align with established quality control limits indicates that the test adheres to industry standards for reproducibility, enhancing its suitability for precise diagnostic applications.

The precision of the assay under regulated and ideal circumstances is the main emphasis of the repeatability test. This evaluation is crucial as it gauges the method's capability to produce consistent results when analyzing the same sample repeatedly.

In examining the repeatability across three levels (low, medium, and high), 30 values were scrutinized for each level, revealing remarkably low coefficients of variation (CV): CV1 = 2.17%, CV2 = 2.04%, and CV3 = 1.38%. These values indicate a small degree of variability, underscoring the high precision of the assay.

The extremely low CV values highlight the assay's outcomes as highly stable and predictable when operating under controlled conditions. Such precision is of utmost importance in clinical testing, where even minor variations can carry significant implications for patient care.

The Mohammed VI University Hospital's central laboratory in Oujda has implemented a quality strategy incorporating a method verification protocol. Conducting this type of investigation will enable the establishment of a credible accreditation process for the analyses conducted in our laboratory. As a pivotal reference center in the Eastern region of Morocco, our laboratory serves not only the needs of referred or hospitalized patients but also contributes to assessing the overall health of the region's general population through various scientific studies (11) ,(12).

## 5. Conclusion

Medical biology has become the cornerstone of the healthcare system, transforming the selection of analysis methods from arbitrary choices to a process guided by specific criteria rooted in the principles of the technique and its validation or verification processes.The Mohammed VI University Hospital's central laboratory is fully dedicated to the accreditation process, and method validation/verification stands out as a crucial step in this commitment (3). The overall reproducibility and repeatability results achieved are outstanding, meeting the stipulations set by RICOS and adhering to the criteria outlined in the Valtec protocol (FSCB). These findings strongly affirm the robustness and reliability of the serum CA 125 assay. This study illustrates the rigorous quality control procedures implemented in medical laboratories and contributes to the indispensable knowledge base required to ensure the accuracy of serum CA 125 measurements. Consequently, it enhances the clinical applications of this assay.

## Compliance with ethical standards

### *Disclosure of conflict of interest*

No conflict of interest to be disclosed.

### *Statement of ethical approval*

Ethical committee approval was unnecessary due to the nature of the article.

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