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(RESEARCH ARTICLE)

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Verification of analytical performance of the C- peptide assay on the Abbott Alinity ci®: Experience of the central laboratory of Mohammed VI University Hospital of Oujda

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Abstract

The objective of our study was to assess the analytical performance of C- peptide determination using a two-step immunoassay with microparticle chemiluminescence immunoassay (CMIA) technology, adhering to Scope A criteria outlined in the guide for the verification/validation of medical biology methods.

We analyzed the C- peptide assay's intermediate precision and reproducibility. For the three levels (low, medium, and high), the obtained results are very satisfactory. For intermediate fidelity, the coefficients of variation are 2.52%, CV2 = 3.70%, and CV3 = 4.37%, respectively. For repeatability, the results): CV1 = 3.15%, CV2 = 1.65%, and CV3 = 1.55%, respectively.

The obtained results enabled verification of method performance and comparison with predefined analytical objectives to ensure compliance with regulatory and normative standards. The central laboratory at University Hospital Mohammed VI of Oujda adheres to a quality policy focused on mastering various analytical systems.

Keywords: C- peptide assay; Analytical performance; Repeatability; Reproducibility Biochemistry laboratory; Abbott Alinity CI analyzer; Chemiluminescent microparticle immunoassay method

1. Introduction

Type 1 diabetes is a condition resulting from an autoimmune attack on the insulin-producing beta cells in the endocrine pancreas. Diagnosis of diabetes occurs when the secretory capacity of the pancreas is greatly reduced (>80%). This corresponds to a significant reduction in the volume and function of beta cells. The beta cells of the pancreatic islets of Langerhans secrete a precursor of insulin, preproinsulin. This precursor generates proinsulin, which is cleaved within the storage granules into insulin and C-peptide. The measurement of C-peptide is widely accepted as the most appropriate measure of residual beta-cell function since it is secreted in equimolar quantities with insulin and, unlike insulin, it is not eliminated during its first pass through the liver. C-peptide has been reported to exert multiple beneficial roles against diabetic complications in human diabetics [1, 2].

The verification of an analytical method in a medical laboratory is essential to ensure that the results closely match the true reference value of a sample, thereby guaranteeing measurement accuracy and reliability. This process involves several steps to comply with the quality standards set by ISO 15189. It includes evaluating the effectiveness of the analytical procedure, measuring its performance through a standardized operational approach, and comparing it

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against predefined benchmarks. Incorrect implementation of these methods can lead to faulty conclusions about method performance, potentially jeopardizing patient safety or leading to incorrect diagnoses [3].

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

1.1. Reminder on C-peptide

C-peptide was discovered in 1967 as a by-product of insulin biosynthesis. Consisting of 31 amino acids, it facilitates the folding of proinsulin. Unlike insulin, C-peptide bypasses first-pass metabolism and circulates at higher levels, with a longer half-life of approximately 30 minutes, primarily excreted by the kidneys. Initially considered inert, recent studies have revealed its significant physiological roles and therapeutic potential, especially in managing chronic diabetes complications such as nephropathy, neuropathy, and retinopathy [4, 5, 6].

Current diabetes treatments control glucose but do not prevent complications, suggesting that other factors, such as C-peptide deficiency, contribute to their development. Restoring C-peptide levels through pancreas or islet transplantation has shown protective effects. C-peptide biosynthesis starts with preproinsulin, which is processed into proinsulin and then cleaved into insulin and C-peptide in the pancreatic beta cells, as depicted in Figure 1. Released alongside insulin in response to glucose, C-peptide plays active biological roles, although its receptor remains unidentified [5].

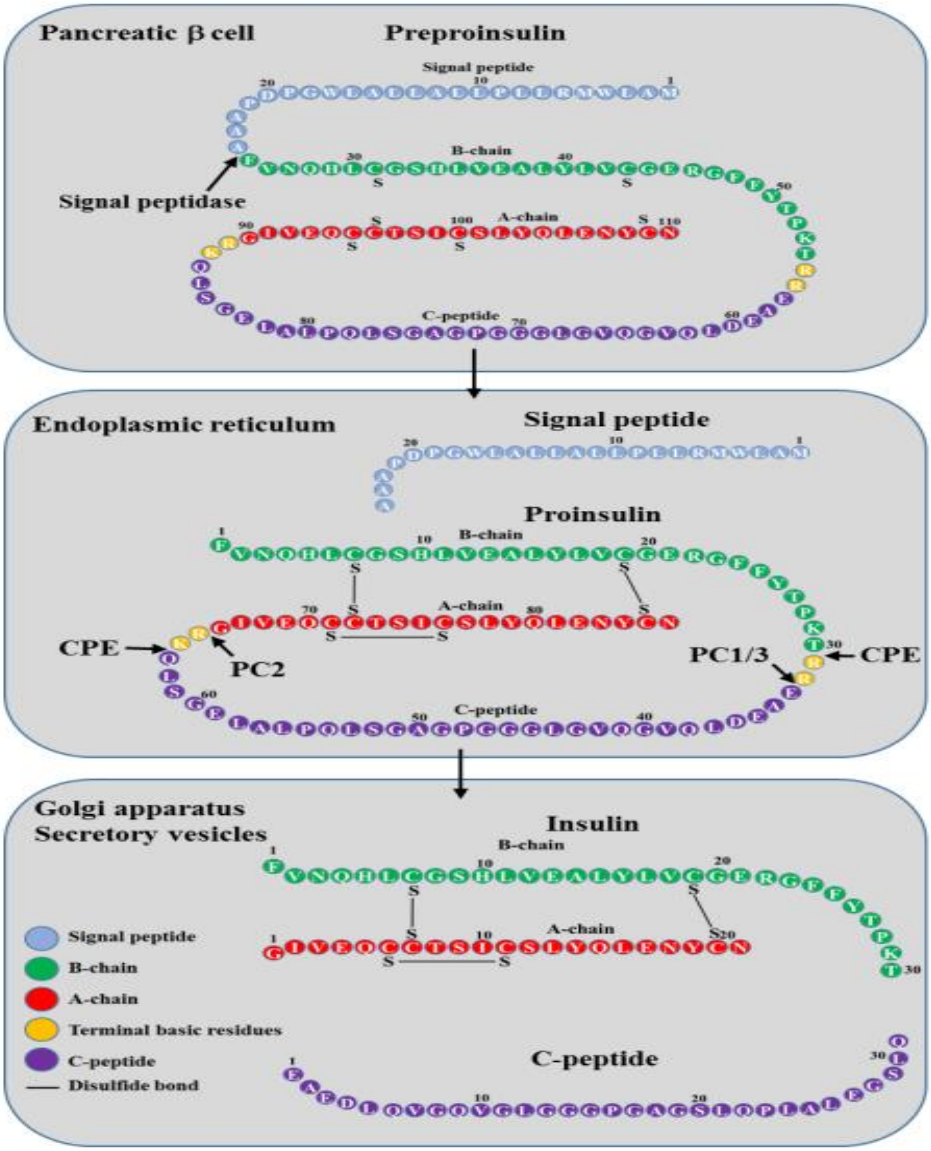

Figure 1 Enzymatic processing and secretion of insulin and C-peptide [5]

Structurally, C-peptide exhibits significant interspecies variability but shows conserved regions essential for its activity, suggesting it functions as more than just a linking segment in insulin biosynthesis. Studies indicate that C-peptide can stimulate specific cellular activities despite its structural variability, supporting its potential as a bioactive peptide with therapeutic applications in diabetes management [2, 5].

C-peptide demonstrates a wide array of therapeutic effects against diabetic complications in both human diabetics and experimental animal models, as depicted in Figure 2. Notably, it enhances glucose utilization, thereby aiding in improved metabolic regulation. In diabetic conditions, C-peptide plays a critical role in enhancing kidney function and reducing structural nerve damage. Moreover, it improves circulation by addressing endothelial dysfunction and enhancing blood flow in coronary arteries and other tissues. C-peptide also shows potential in reducing diabetic macular edema and protecting against retinal damage, underscoring its versatility as a therapeutic agent in managing diabetes [5].

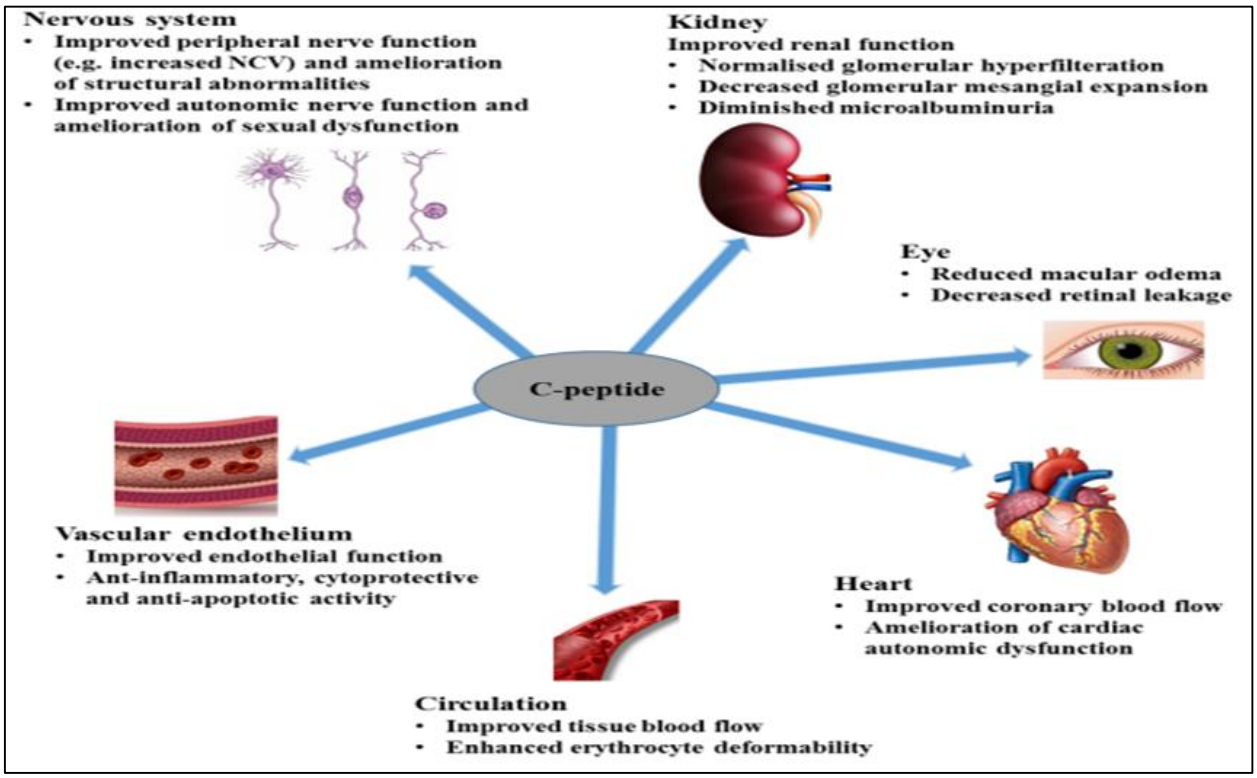

Figure 2 Multiple actions of C-peptide in diabetes [5]

1.2. Principle of C-peptide assay method

This assay is a two-step immunoassay for the quantitative determination of C-peptide in human urine, serum, and plasma, utilizing chemiluminescent microparticle immunoassay (CMIA) technology. The sample, paramagnetic microparticles coated with anti-human C-peptide antibodies, and the assay diluent are combined and incubated. The C-peptide present in the sample binds to the anti-human C-peptide antibody-coated microparticles. After washing, the acridinium-labeled anti-human C-peptide antibody conjugate is added to form a reaction mixture, which is then incubated. Following another wash cycle, the pre-trigger and trigger solutions are added [7].

The resulting chemiluminescent reaction is measured in relative light units (RLUs). There is a direct relationship between the amount of C-peptide present in the sample and the RLUs detected by the optical system.

2. Materials and methods

This study is a prospective investigation conducted within the biochemistry laboratory of Mohammed VI University Hospital, spanning a duration of 30 days. The working methodology is based on the recommendations of the French accreditation committee (COFRAC) accreditation technical guide GTA 04. The study was structured around two distinct phases.

The initial phase involved evaluating the reproducibility of results. This was achieved through daily testing of control samples at three concentration levels—low, medium, and high—over the course of 30 days. The primary aim was to assess the consistency and reliability of the assay.

In the subsequent phase, a comprehensive collection of serum samples was amassed, ensuring an equitable distribution of C-peptide values across the full measurement spectrum. These collected samples were categorized into three groups representing low, medium, and high C-peptide levels. To gauge repeatability, each serum sample underwent 30 individual assay runs.

The C-peptide determination was conducted utilizing a dedicated reagent kit on the immunology module of the Abbott Alinity CI analyzer. Subsequent data processing was carried out via the BYG middleware, which served as an intermediary software bridging the gap between the Alinity platform and the iLab result validation software. The coefficient of variation (CV) values yielded by this study were subsequently compared against the standards stipulated by established learned societies, namely the Federation of Clinical Chemistry and Laboratory Medicine (FSCB).

The results of this analysis are presented in the subsequent sections.

3. Results

3.1. Intermediate fidelity results

Intra-laboratory reproducibility, also known as 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 the results. The data is used to calculate the mean, standard deviation, and coefficient of variation (CV%) for each series, within series, between series, and for all data.

The intermediate fidelity outcomes were acceptable across the three levels—low, medium, and high—with coefficients of variation (CV) of 2.52%, 3.70%, and 4.37%, 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 are graphically depicted through Levey-Jennings plots (Fig. 3, Fig. 4, and Fig. 5) to enhance the clarity of the findings.

Table 1 Reproducibility results of blood assay by level with comparison to FSBC

Level of IQC	Numbers of value	Mean (pg/l)	Standard deviation	Coefficient of variation CV (%)	Reference CV: FSBC 1999
Low	30	0.88	0.022	2.52%	15.0%
Medium	30	3.73	0.138	3.70%	8.0%
High	30	7.45	0.326	4.37%	8.0%

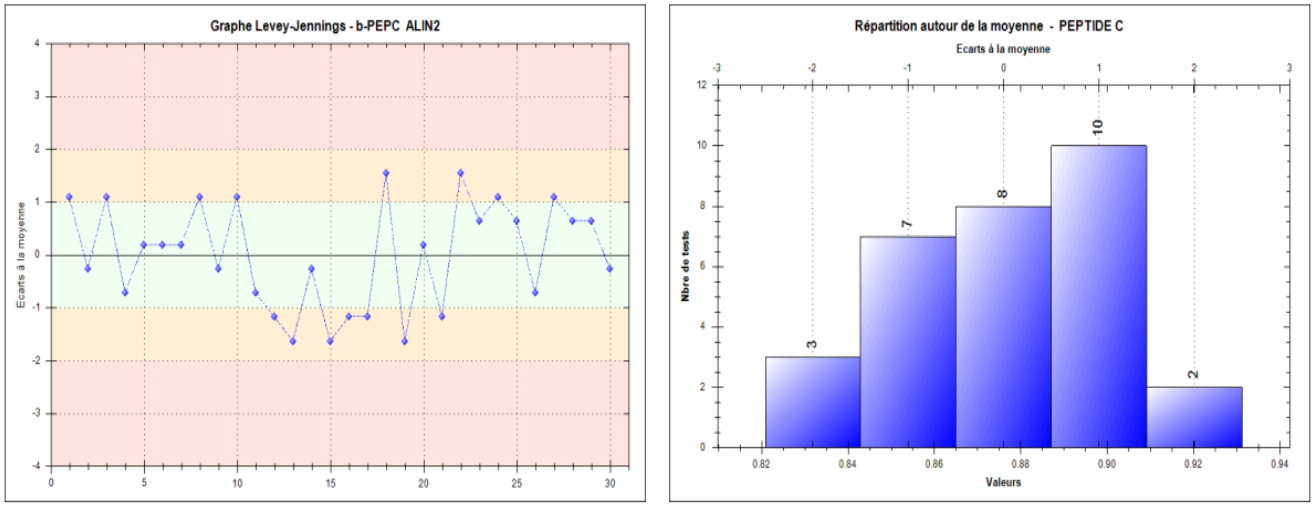

Figure 3 Low Level of reproducibility: Levey Jennings graph and the distribution around the mean

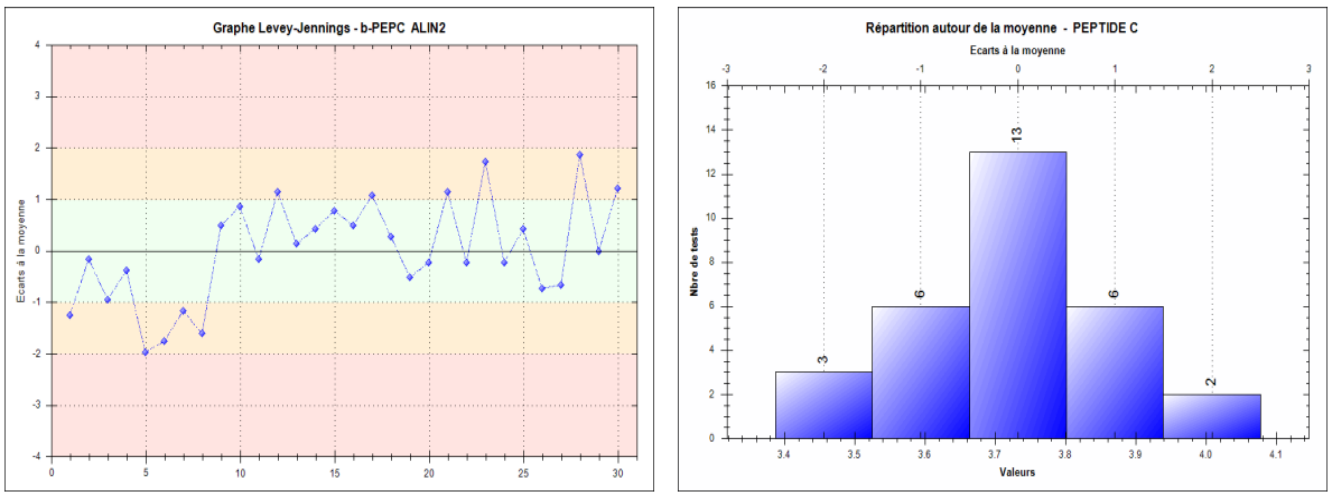

Figure 4 Medium Level of reproducibility: Levey Jennings graph and the distribution around the mean

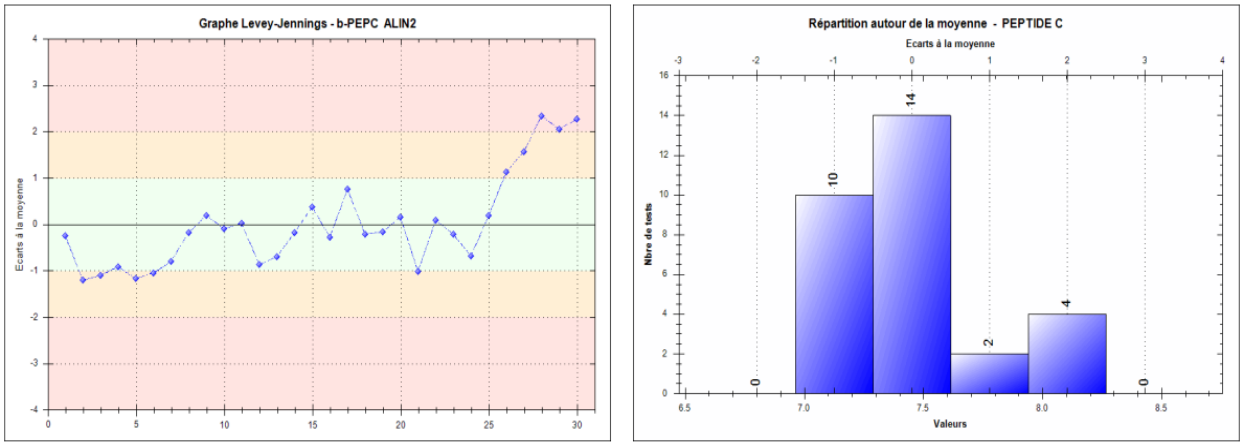

Figure 5 High Level of Reproducibility: Levey Jennings graph and the distribution around the mean

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 reagents, calibration, instrument, and operator,

within the briefest time frame possible. The repeatability test enables the initial performance to be determined and verifies the correct operation of the system (instrument/reagent) for the analyte concerned. Once more, variability is measured using CV values. As indicated in Table 2, the results obtained for the various C-peptide assay verification criteria demonstrate satisfactory repeatability for all three levels—low, medium, and high—with coefficients of variation (CV) of 3.15%, 1.65%, and 1.55%, respectively, on 30 samples.

Table 2 Repeatability results for C-peptide on the Alinity i® automated system by level with comparison to SFBC

Level of IQC	Number of value	Mean (pg/l)	Standard deviation	Coefficient of variation CV (%)	Reference CV: FSBC 1999
Low	30	0.89	0.028	3.15 %	11.25%
Medium	30	3.58	0.059	1.65%	6.0%
High	30	7.64	0.119	1.55 %	6.0%

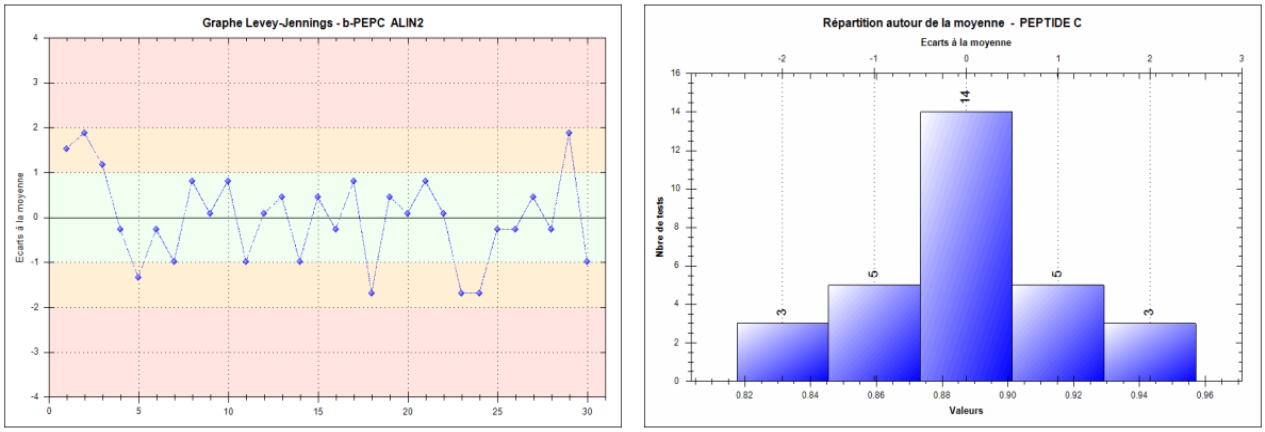

Figure 6 Low Level of Repeatability: Levey Jennings graph and the distribution around the mean

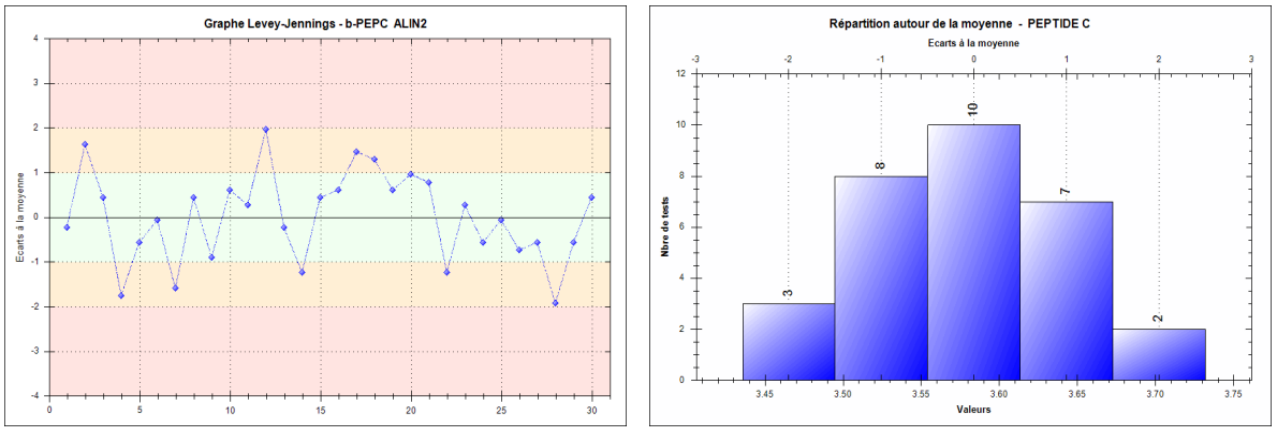

Figure 7 Medium Level of Repeatability: Levey Jennings graph and the distribution around the mean

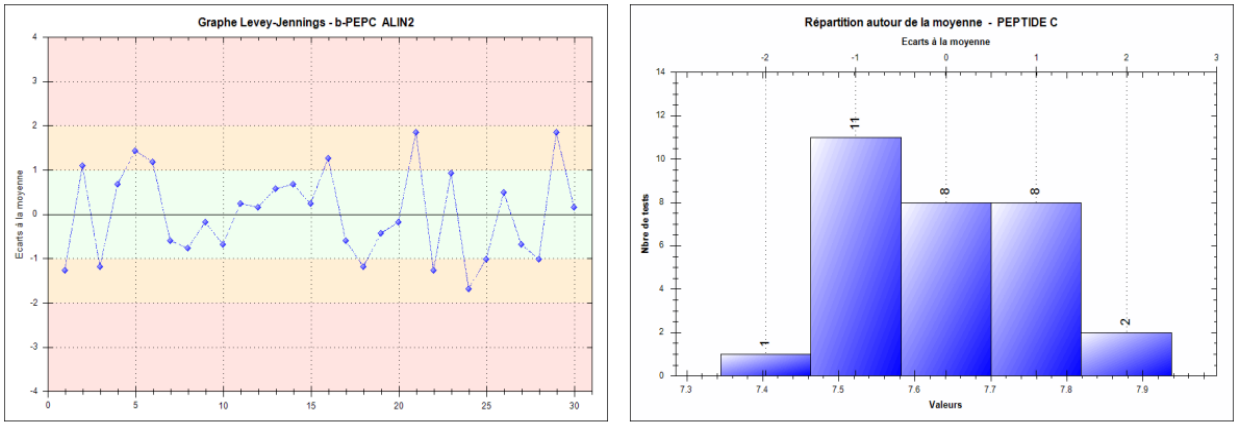

Figure 8 High Level of Repeatability: Levey Jennings graph and the distribution around the mean

4. Discussion

C-peptide is indeed a byproduct of proinsulin conversion to insulin within pancreatic beta cells, released into the bloodstream in roughly equimolar amounts alongside insulin. This close correlation between insulin and C-peptide levels serves as a reliable marker for assessing endogenous insulin secretion. However, exceptions exist, particularly in obese individuals and those with islet cell tumors [5, 8].

The diagnosis of hypoglycemia due to surreptitious insulin administration can be supported by measuring serum C-peptide levels. In cases where exogenous insulin is the cause of hypoglycemia, serum C-peptide levels will be low because synthetic insulins used in treatment do not contain C-peptide. This discrepancy helps differentiate between endogenous insulin secretion and exogenous insulin administration [9].

High levels of C-peptide can be observed when the activity of beta cells is exacerbated, as in the cases of hyperinsulinism and insulinomas [5].

The verification and validation of methods within a medical laboratory is a critical process that ensures precise and reliable measurements. This process not only meets regulatory requirements outlined in The Moroccan Guide for the Good Performance of Medical Laboratory Analysis (GBEA) but also adheres to the ISO 15189:2022 standards [10, 11]. The C-peptide assay, which uses the immunochemiluminescent method, is currently validated, requiring verification rather than validation. In this study, we verified the analytical performance of the C-peptide assay on the Abbott Alinity CI analyzer using the COFRAC guide SH-GTA-04. Repeatability and intermediate fidelity are key statistical methods in process control, measuring precision and variability within automated systems [12].

The intermediate fidelity test, also known as intra-laboratory reproducibility, involves examining a single sample under various conditions by altering at least one factor, such as the operator, timing, reagent kits, or calibrations. This process helps establish acceptance criteria in line with prior data, considering biological variations, and is especially valuable in decision support systems [13, 14]. Our study confirmed the reliability of the C-peptide assay for reproducibility assessment, with three levels—low, medium, and high—yielding satisfactory outcomes. For each level, 30 values were analyzed, showing means of m1 = 0.88 (pg/l), m2 = 3.73 (pg/l), and m3 = 7.45 (pg/l), with coefficients of variation (CV) of CV1 = 2.52%, CV2 = 3.70%, and CV3 = 4.37%.

The results of intermediate fidelity evaluation suggests that the C-peptide assay, employing the immune-chemiluminescent method, has shown consistency and agreement in measurements across different conditions. Which suggest that the technique is robust and reliable, and it can be trusted for clinical diagnosis.

The repeatability test evaluates the precision of an assay under controlled and optimal conditions, emphasizing its ability to produce consistent results when the same sample is analyzed multiple times. This assessment is crucial for ensuring the reliability of the method in yielding similar outcomes under regulated and ideal circumstances [15, 16].

The repeatability test showed remarkably low coefficients of variation across three levels (low, medium, and high): CV1 = 3.15%, CV2 = 1.65% and CV3 = 1.55%. These values indicate minimal variability, underscoring and reaffirming the high precision of the assay.

The immune-chemiluminescent C-peptide assay shows high precision in repeatability, with consistently low coefficients of variation (CVs). This indicates minimal variability in repeated measurements under identical conditions, emphasizing the method's reliability and robustness. Such precision is critical in clinical testing, ensuring consistent and accurate results.

The central laboratory at Mohammed VI University Hospital in Oujda has implemented a quality strategy encompassing a method verification protocol. Conducting this study type is pivotal in establishing a robust accreditation process for analyses conducted in our laboratory. Positioned as a reference center in the Eastern region of Morocco, our laboratory not only caters to referred or hospitalized patients but also plays a vital role in evaluating the health of the broader regional population through diverse scientific studies. This strategic approach ensures the reliability of our laboratory processes, reinforcing its significance in contributing to healthcare excellence and scientific research endeavors in the region [17, 18, 19, 20].

5. Conclusion

The study revealed satisfactory results, meeting the supplier's criteria and the FSBC protocol, and complying with the ISO 15189 standard. The CMIA method showed strong reproducibility and repeatability, with low Coefficient of Variation (CV) values, indicating consistent and stable performance. These results aligned with industry standards, confirming the method's reliability for serum C-peptide assays in clinical settings. This contributes valuable insights to clinical diagnostics, enhancing the accuracy and dependability of diagnostic processes for improved patient care.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declares that they have no known competing financials interests or personal relationships that could have appeared to influence the work reported in this paper.

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