

Verification of the analytical performance of the serum total cholesterol assay on Abbott Architect ci8200

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Abstract

Serum total cholesterol measurement is an essential aspect of preventive healthcare as high levels of cholesterol are linked to various diseases such as atherosclerosis, heart attacks, and strokes. Serum cholesterol measurement is a simple blood test that assesses the levels of cholesterol in the bloodstream. It is an important diagnostic tool that helps healthcare professionals evaluate a patient's risk of developing cardiovascular disease and make decisions regarding treatment and lifestyle changes.

Our study aims to verify the analytical performance of the serum total cholesterol assay method using an Abbott kit on the Architect ci8200 automated system in the biochemistry laboratory of the Mohammed VI University Hospital of Oujda. The analytical performance of the kit was verified through flexible scope A by performing a performance study on the Architect ci8200. We evaluated the repeatability, the reproducibility, the imprecision of the measurements and the comparison between two Architect ci8200 automatons. All the results obtained are in conformity with the acceptability criteria recommended by the supplier and the Valtec protocol of the 'Société Française de Biologie Clinique' (SFBC), and the study is globally satisfactory.

The analytical performance of the Architect ci8200 automated system was satisfactory for a reliable determination of total cholesterol. Verification of methods of dosage in the medical laboratory is crucial to ensure the accuracy, precision, and reliability of laboratory test results. Verification involves confirming that the test method employed is appropriate for the intended use, produces results that are consistent with the claimed performance characteristics, and meets the laboratory's quality control and quality assurance requirements.

Keywords: Total cholesterol; Verification; Quality; Architect ci 8200; Repeatability; Reproducibility

1. Introduction

Method verification in the field of laboratory analysis involves evaluating the performance of an analytical process through a standardized protocol.[1] This evaluation is based on predetermined criteria set by respected organizations such as RICOS and SFBC [2], [3]. This process provides laboratories with a comprehensive understanding of their analysis methods, performance capabilities, and limitations. The objective is to ensure that these performances are adequate to guarantee the accuracy and reliability of analytical results and their clinical interpretations, benefiting both patients and healthcare providers.

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Serum total cholesterol is one of the parameters measured in our laboratory on the Abbott Architect ci 8200 automated system. In order to ensure the quality of our serum total cholesterol results, it was of paramount importance to commit to the procedure of the verification of its determination method.

Our study aims to verify the analytical performance of the serum total cholesterol assay method using an Abbott kit on the Architect ci8200 automated system in the biochemistry laboratory of the Mohammed VI University Hospital of Oujda. The verification of the analytical performance of the kit was carried out through flexible scope A by performing a performance study on the Architect ci8200. We evaluated the repeatability, the reproducibility, the imprecision of the measurements and the comparison between two Architect ci8200 automatons. All the results obtained are in conformity with the acceptability criteria recommended by the supplier and the Valtec protocol of the 'Société Française de Biologie Clinique' (SFBC), and the study is globally satisfactory.

1.1. Interest of serum total cholesterol determination

Serum total cholesterol measurement is an essential aspect of preventive healthcare as high levels of cholesterol are linked to various diseases such as atherosclerosis, heart attacks, and strokes[4-5]. Several meta-analyses have studied the association between total cholesterol levels and the risk of death from cardiovascular disease.[6]

Total cholesterol levels play a significant role in predicting mortality from cardiovascular disease. The novel SCORE2 algorithm, incorporates additional risk factors, including total cholesterol levels, to enhance risk stratification.[7]

Serum cholesterol measurement is a simple blood test that assesses the levels of cholesterol in the bloodstream. It is an important diagnostic tool that helps healthcare professionals evaluate a patient's risk of developing cardiovascular disease and make decisions regarding treatment and lifestyle changes. Monitoring cholesterol levels is particularly important for individuals with a family history of heart disease, high blood pressure, or diabetes.

1.2. Princip of the assay method

Cholesterol esters are enzymatically hydrolyzed by cholesterol esterase, which breaks them down into free cholesterol and fatty acids. The free cholesterol, including the initially present one, is then oxidized by cholesterol oxidase to form cholesten-4-one-3 and hydrogen peroxide. Hydrogen peroxide combines with hydroxybenzoic acid (HBA) and 4-aminoantipyrine to form a chromophore (quinoneimine) quantified at 500 nm (**Table 1**). The intensity of the coloration is proportional to the amount of H₂O₂ formed and, therefore, to the amount of free cholesterol.

Table 1 Reagent composition

Reactive Ingredients	Concentration
Cholesterol Oxidase (Microbial)	> 200 U/L
Cholesterol Esterase (Microbial)	> 500 U/L
Peroxidase (Horseradish)	> 300 U/L
4-Aminoantipyrine	<0.5 mmol/L
hydroxybenzoic acid (HBA)	10 mmol/L

2. Material and methods

This is a prospective study that was conducted in the biochemistry laboratory of the Mohammed VI University Hospital, over a period of 36 days.

Our study was divided into two parts. The first step concerned the assessment of reproducibility through the daily passing of control of the three levels—low, medium, and high—over a period of 36 days. The serum samples were gathered in the second stage, and the total cholesterol readings were uniformly spread across the measurement range. Three groups of samples were created: low, medium, and high levels. The Cholesterol kit ran the test on the Chemistry module. The BYG middleware, a gateway program between the Architect ci8200 and the iLab result validation software, processed data. The CV values obtained by our study were compared with those set by the learned societies (SFBC and RICOS).

Furthermore, we conducted a method comparison between the two automated systems Architect ci8200®, employing the Bland-Altman diagram to visualize and analyze the differences in results obtained from these techniques in relation to their respective means.

3. Results

3.1. Intermediate fidelity results

The intermediate fidelity test, also known as intra-laboratory reproducibility, involves analyzing the same sample under diverse conditions, where at least one variable is altered, such as the operator, time, reagent batches, or calibrations.[8] This approach facilitates the establishment of acceptance criteria based on prior knowledge, taking into account biological variations, especially in the context of decision support systems. By subjecting the sample to various conditions and meticulously observing the resultant outcomes, researchers can discern the influence of different factors on the test's accuracy and reliability. This process contributes to a comprehensive understanding of the test's robustness and performance, aiding in the development and optimization of diagnostic methodologies and enhancing the overall quality of laboratory analyses in the field of clinical diagnostics[9].

The intermediate fidelity results demonstrated a high level of satisfaction across all three levels: low, medium, and high, with a coefficient of variations (CV) of 1.2%, 2.17%, and 2.9%, respectively. These outcomes have been visually represented through Levey-Jennings graphs, as shown in Figure 1, Figure 2 and Figure 3.

The conclusion drawn from the intermediate fidelity results for the low, medium, and high levels of analysis reveals a meticulous evaluation of the coefficient of variation (CV) of Reproducibility, expressed as percentages. These CV values are compared against the appropriate FSBC and RICOS limits, which include expansion factors ($k = 1.219$ for low, $k = 1.223$ for medium, and $k = 1.232$ for high).

3.1.1. Low Level

The FSBC limit with the expansion factor ($k = 1.219$) is determined to be 4.88%, while the RICOS limit with the same expansion factor is 3.29%. The CV of Reproducibility (1.2) at the low level is observed to be correct and well below the tolerated limit, ensuring a high level of precision and reliability in the measurements at this level.

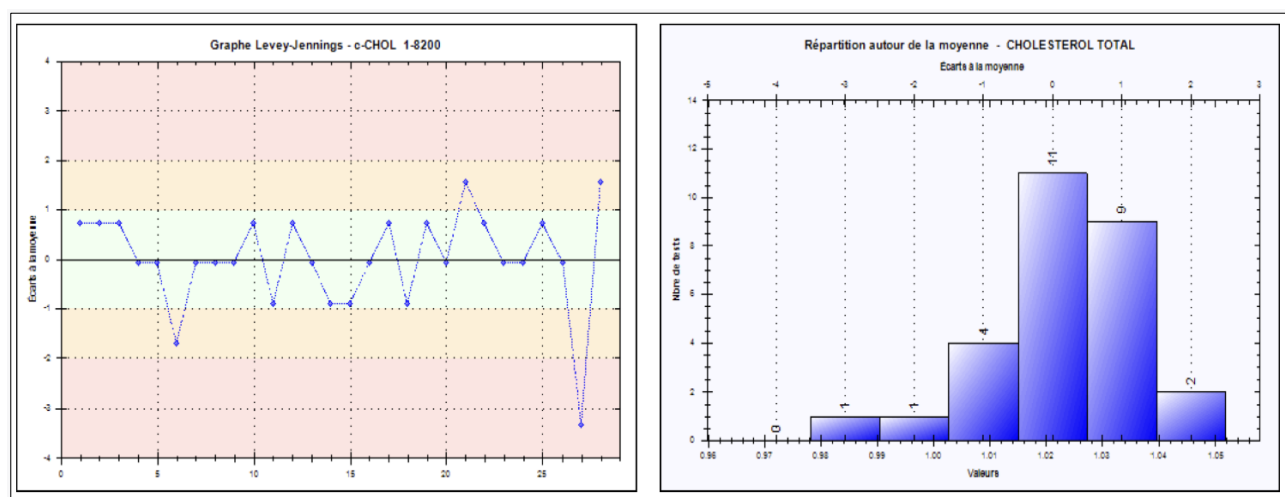


Figure 1 Low Level of Reproducibility: Levey Jennings graph and the distribution around the mean – Total serum cholesterol

3.1.2. Medium Level

For the medium level, the FSBC limit with the expansion factor ($k = 1.223$) is found to be 4.89%, and the RICOS limit with the expansion factor is 3.3%. In this case, the CV of Reproducibility (2.17) is deemed correct, as it falls below the tolerated limit, further validating the precision and reproducibility of the assay at this level.

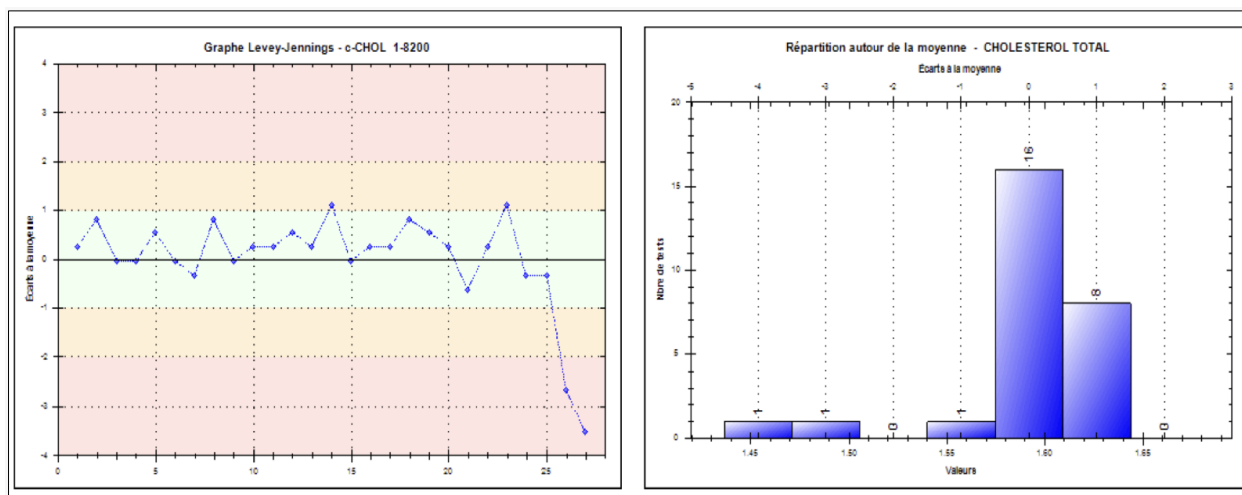


Figure 2 Medium Level of Reproducibility: Levey Jennings graph and the distribution around the mean – Total serum cholesterol

3.1.3. High Level

At the high level of analysis, the FSBC limit with the expansion factor ($k = 1.232$) is calculated to be 4.93%, whereas the RICOS limit with the expansion factor stands at 3.33%. Once again, the CV of Reproducibility (2.9) is considered correct, as it remains below the tolerated limit, attesting to the assay's accuracy and reliability even at this demanding level of analysis.

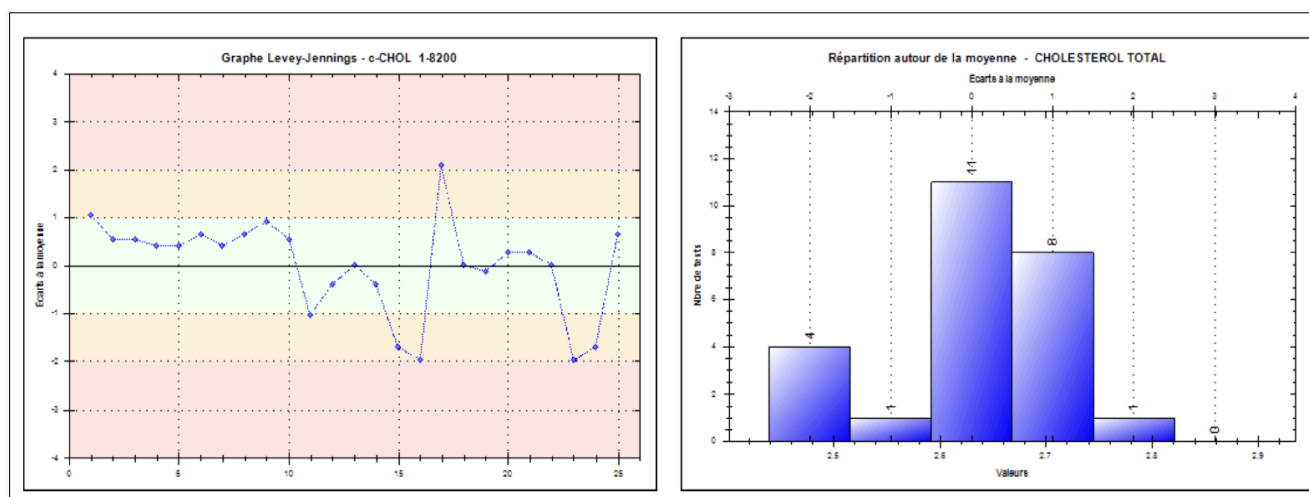


Figure 3 High Level of Reproducibility: Levey Jennings graph and the distribution around the mean – Total serum cholesterol

3.2. Repeatability results

Repeatability testing involves subjecting the same sample to a series of analyses under specific conditions, namely the same operator, the same batch of reagents, the same instrument, and the same calibration, all performed in the shortest feasible timeframe. The overarching objective is to meticulously assess the analyte's performance under optimal conditions and to verify the proper functioning of the system, encompassing the instrument and reagents. This assessment serves as a crucial step in ensuring the accuracy and reliability of the analytical process for the specific analyte of interest.[8], [10]

For each analyte/matrix combination to be measured on a particular analyzer, the repeatability calculation must be carried out, considering multiple concentration levels. These chosen levels are strategically aligned with distinct medical decision areas. The calculated coefficient of variation (CV) is then meticulously compared against a pre-established acceptable limit CV, previously determined based on specific quality and accuracy criteria.

The outcomes derived from the repeatability testing exhibited commendable performance across all three concentration levels: low, medium, and high. The CV values obtained were as follows: CV1 = 1.78%, CV2 = 1.15%, and CV3 = 1.14% respectively. These results affirm a satisfactory level of repeatability, indicative of the method's precision and reliability, even under varying concentration levels.

To visually represent these exemplary findings, Levey Jennings graphs were constructed, effectively demonstrating the consistent and robust performance of the analytical process. These graphical representations, as shown in Figure 4, Figure 5, and Figure 6, provide a comprehensive visualization of the method's accuracy and precision across the range of analyzed concentrations.

The conclusion drawn from the repeatability testing showcases a meticulous assessment of the coefficient of variation (CV) of Repeatability for the low, medium, and high concentration levels. The CV values are compared against the established SFBC and RICOS limits, which include expansion factors ($k = 1.215$ for low, and $k = 1.208$ for medium and high).

3.2.1. Low Level

For the low concentration level, the SFBC limit with the expansion factor ($k = 1.215$) is determined to be 3.65%, while the RICOS limit with the same expansion factor stands at 2.46%. The CV of Repeatability (1.78) obtained at this level is found to be correct, as it remains below the tolerated limit. This exemplary precision demonstrates the method's ability to consistently deliver reliable results, even at lower analyte concentrations, thereby instilling confidence in the assay's clinical utility.

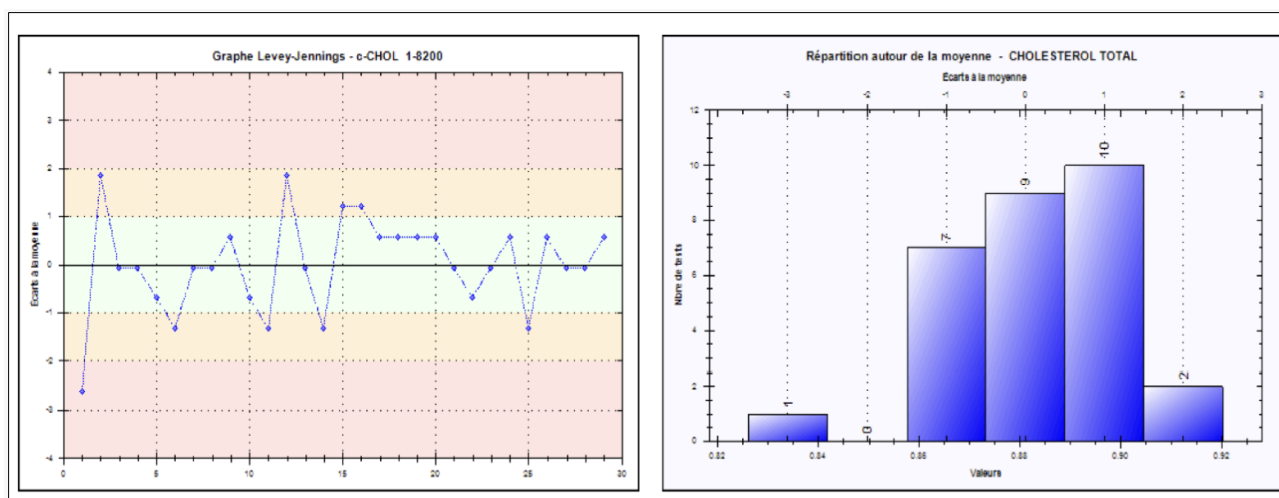


Figure 4 Low level of repeatability: Levey Jennings graph and the distribution around the mean – Total serum cholesterol

3.2.2. Medium Level

At the medium concentration level, the SFBC limit with the expansion factor ($k = 1.208$) is calculated to be 3.62%, while the RICOS limit with the same expansion factor is 2.45%. In this case, the CV of Repeatability (1.15) is confirmed to be correct, as it falls below the accepted limit, underscoring the assay's robust performance and reproducibility at intermediate analyte concentrations.

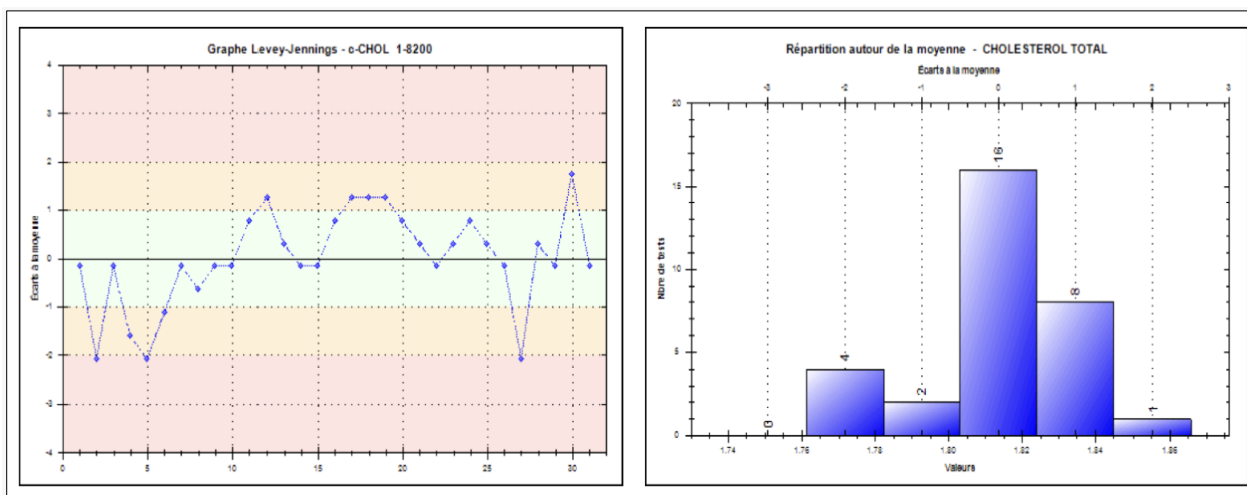


Figure 5 Medium level of Repeatability: Levey Jennings graph and the distribution around the mean – Total serum cholesterol

3.2.3. High Level

The high concentration level yields similar results, with the SFBC limit and RICOS limit both determined to be 3.62% and 2.45%, respectively. The CV of Repeatability (1.14) at this level is deemed correct, further emphasizing the method's precise and reliable performance even under challenging conditions.

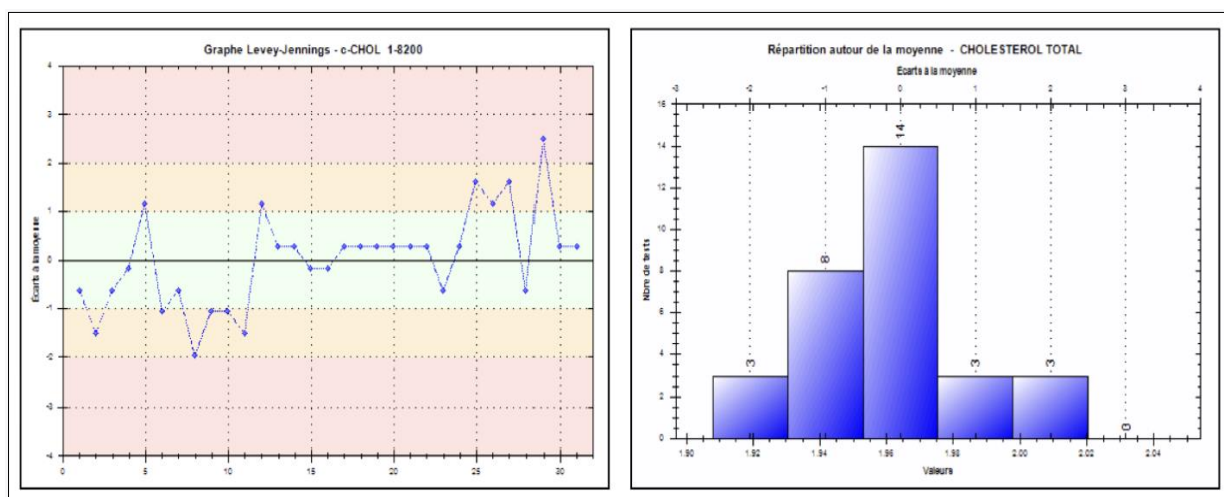


Figure 6 High level of Repeatability: Levey Jennings graph and the distribution around the mean – Total serum cholesterol

We conducted a method comparison study using 29 samples to assess the agreement between two automats. The Bland-Altman diagram (Figure 7) revealed an average bias of approximately -0.156 %, and a linear regression equation $Y = 1 X + 0.002$ was derived from the analysis. The mean of the differences between the two automata was -0.002 g/l, with a standard deviation of 0.016 g/l. (Figure 8)

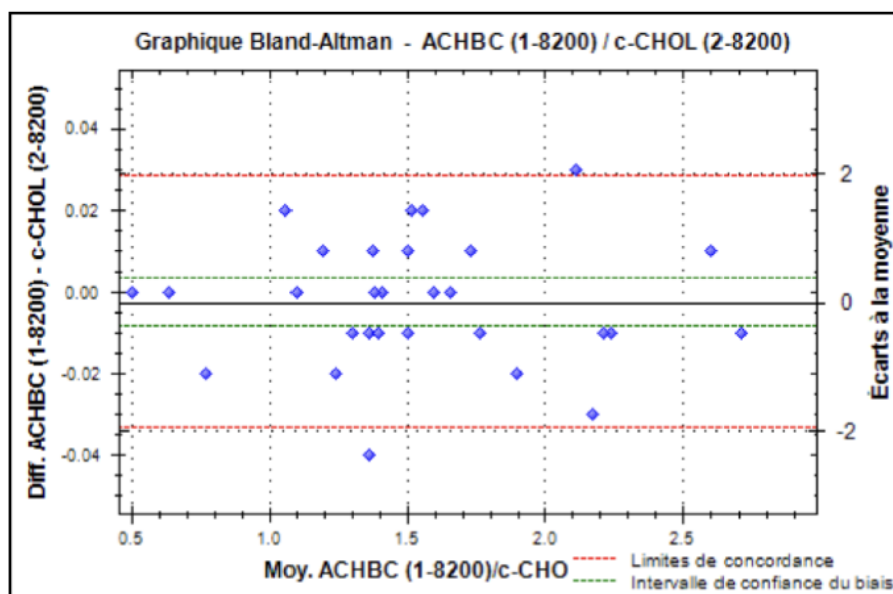


Figure 7 The Bland-Altman diagram for serum total cholesterol

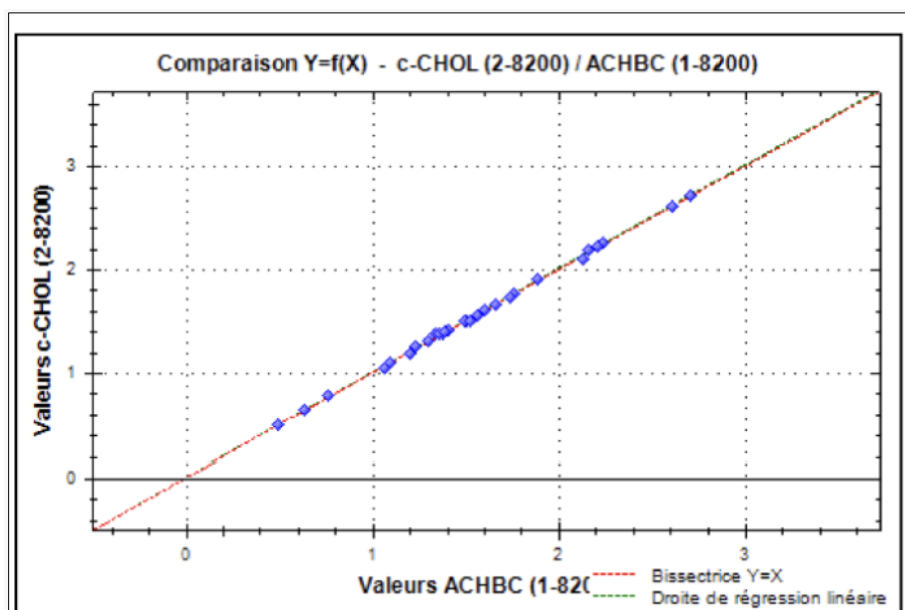


Figure 8 Serum total cholesterol method comparison result between two Architect ci8200®

Furthermore, we evaluated the measurement uncertainty for different levels of the Abbott Architect ci8200® instrument, and the results were within acceptable ranges. Specifically, the measurement uncertainties for levels 1, 2, and 3 were determined to be 7.52%, 7.73%, and 8.13%, respectively, as summarized in Table 2.

Table 2 Measurement uncertainty results for total serum cholesterol on Architect ci8200 automaton

Sample	measurement uncertainty	Performance requirement	Conclusion
Level 1	7.52 %	8.5 %	Validated
Level 2	7.73 %	8.5 %	Validated
Level 3	8.13 %	8.5 %	Validated

4. Discussion

We undertook the process of validating and verifying our method according to the quality requirements outlined in scope A of the NF ISO 15,189 standard, adhering to the guidelines specified in the COFRAC guide SH-GTA-04. Our primary goal is to ensure the reliability and accuracy of our test results. Method validation and verification are crucial steps in the accreditation process, to which our laboratory is fully committed. [11–13]

The outcomes of our study robustly demonstrated the reliability of the total cholesterol assay results when evaluating reproducibility across three concentration levels, all of which yielded satisfactory results (*Table 3*). For the low concentration level, 28 values were meticulously analyzed, with a mean of 1.02 g/l and a coefficient of variation (CV) of 1.2% (*Figure 1*). Similarly, at the medium concentration level, 27 values were analyzed, showing an average of 2.59 g/l with a CV of 2.17% (*Figure 2*). At the high concentration level, 25 values were analyzed, revealing a mean of 2.63 g/l with a CV of 2.9% (*Figure 3*).

The meticulous comparison of the CV values against the established limits underscores the robustness and precision of the analytical method across the entire range of analysis. The findings affirm the method's capability to consistently deliver accurate and reproducible results, fulfilling the stringent requirements of clinical diagnostics. This comprehensive evaluation of intermediate fidelity provides a robust basis for integrating the method into clinical decision support systems and underscores its suitability for use in high-stakes diagnostic scenarios. Moreover, it demonstrates the laboratory's commitment to quality assurance and proficiency, reinforcing the credibility and trustworthiness of the analytical data generated in clinical practice.

Table 3 Reproducibility results obtained on the 3 levels: low, medium and high

Operators evaluated	Number of values	Variability results
Low level	28	Mean: 1.02 g/l
		SD: 0.012 g/l
		CV: 1.2 %
Medium level	27	Mean: 1.59 g/l
		SD: 0.035 g/l
		CV: 2.17 %
High level	25	Mean: 2.63 g/l
		SD: 0.076 g/l
		CV: 2.9 %

The evaluation of repeatability also yielded compelling results. At the low concentration level, 29 values were subjected to analysis, resulting in a mean of $m_1 = 0.88$ g/l and an impressively low CV of 1.78%. The medium concentration level was equally impressive, with 31 values analyzed, leading to an average of $m_2 = 1.81$ g/l and a CV of 1.15%. Lastly, for the high concentration level, 31 values were meticulously analyzed, yielding a mean of $m_3 = 1.96$ g/l with an excellent CV of 1.14%. (Table 4)

Table 4 Repeatability results obtained on the 3 levels: low, medium and high

Samples	Number of values	Mean	SD	CV
Low level	29	0.88 g/l	0.016 g/l	1.78 %
Medium level	31	1.81 g/l	0.021 g/l	1.15 %
High level	31	1.96 g/l	0.022 g/l	1.14 %

The exceptionally low coefficients of variation achieved in both reproducibility and repeatability analyses unequivocally meet the rigorous requirements set forth by esteemed learned societies such as RICOS and SFBC. These low CV values underscore the assay's outstanding precision and reliability across all three concentration levels, even when dealing with challenging analyte concentrations.

The comparison of CV values against the established limits reaffirms the analytical method's excellent repeatability, showcasing its capability to consistently deliver accurate and reproducible results across a wide range of analyte concentrations. The assay's precision, as evidenced through these comprehensive repeatability evaluations, underscores its suitability for integration into medical decision support systems, clinical diagnostic algorithms, and high-stakes diagnostic applications. By consistently achieving CV values below the tolerated limits, the method demonstrates its adherence to rigorous quality control measures, thereby instilling trust in the reliability of clinical data generated and contributing to advancements in medical knowledge and patient care.

The impressive performance of the total cholesterol assay, as evidenced by the meticulous evaluation of reproducibility and repeatability, serves as a testament to the laboratory's unwavering commitment to adhering to stringent quality control measures. The results substantiate the laboratory's adherence to international standards and guidelines, positioning it as a center of excellence in clinical diagnostics and research. By consistently achieving CV values well below the acceptable limits, the laboratory enhances the trust of healthcare providers and patients in the accuracy and reliability of the analytical data generated, ultimately benefiting patient care and advancing the field of clinical diagnostics. These exemplary findings contribute to the ever-evolving landscape of medical knowledge and foster a culture of continuous improvement in laboratory practices, ultimately benefitting the health and well-being of the population served by the Mohammed VI University Hospital.

The central laboratory of the Mohammed VI University Hospital in Oujda has established a comprehensive quality strategy, encompassing a meticulous method verification procedure. Undertaking such a study is pivotal in establishing a robust and reliable accreditation process for the laboratory's analyses. As a center of reference in the Eastern region of Morocco, the laboratory plays a pivotal role in providing diagnostic services not only to referred or hospitalized patients but also in evaluating the overall health of the general population in the region through various scientific studies.[14]–[20].

5. Conclusion

The analytical performance of the Architect ci8200 automated system was satisfactory for a reliable determination of total cholesterol. Verification of methods of dosage in the medical laboratory is crucial to ensure the accuracy, precision, and reliability of laboratory test results. Verification involves confirming that the test method employed is appropriate for the intended use, produces results that are consistent with the claimed performance characteristics, and meets the laboratory's quality control and quality assurance requirements.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest in preparing this article.

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