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(RESEARCH ARTICLE)

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Verification of analytical performance of Complement C4 on the Abbott Alinity ci®: Experience of the central laboratory of Mohammed VI University Hospital of Oujda

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Abstract

The primary objective of our study was to validate the analytical performance of complement C4 assay performed on the Abbott Alinity ci®: analyzer using the Immunoturbidimetric method. This validation was conducted in the biochemistry laboratory at Mohammed VI University Hospital in Oujda. The methodology followed the guidelines outlined in the french accreditation committee (COFRAC) technical guide (GTA) 04, focusing on the evaluation of reproducibility and repeatability.

Overall, the results were satisfactory and met the standards set by both the manufacturer and the French Society of Clinical Biology. This study highlights the capability of the biochemistry laboratory at Mohammed VI University Hospital in Oujda to deliver accurate and reliable results, which are critical for effective clinical diagnosis and decision-making.

Keywords: Complement C4; Verification; Repeatability; Reproducibility; Alinity CI

1. Introduction

Quality is a continuous commitment to consistently delivering accurate test results. A comprehensive quality assurance system integrates all laboratory operations—both internal and external—by adopting effective practices and enhanced management skills. This approach ensures tests are conducted accurately on appropriate samples from correct subjects within well-equipped facilities, enabling reliable interpretations based on precise reference data.

To embed quality principles in medical laboratories, establishing a robust quality management program is vital for ensuring the reliability and integrity of laboratory results. In recent years, significant progress has been achieved in improving clinical laboratory quality, particularly through the adoption of accreditation based on ISO 15189 standards. These standards evaluate both technical and managerial competencies. The accreditation process involves the validation, verification, and continuous quality assurance of testing methods. Accredited laboratories are required to assess and document the analytical performance of all methods, not only before implementation but throughout their operational lifecycle. Clear, standardized, and practical guidelines are essential to support these efforts.

The verification of analytical methods in medical laboratories is crucial to ensure that test results accurately represent the true reference values of samples, thereby ensuring precise and reliable measurements. This process involves a structured approach to comply with the quality standards set by ISO 15189. It includes evaluating the efficiency of analytical procedures, assessing performance through standardized methodologies, and comparing outcomes to established benchmarks. Incorrect implementation of these methods can result in inaccurate evaluations of their performance, potentially jeopardizing patient safety and causing incorrect diagnoses.

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The central laboratory of the Mohammed VI University Hospital in Oujda has implemented a quality strategy that includes a method verification protocol, with our study forming an integral part of this initiative.

Our study focuses on the critical process of method verification for the complement C4 assay assay utilizing immunoturbidimetric technology on the Abbott Alinity Ci analyzer. This process involves evaluating analytical performance, measuring it through standardized operational procedures, and comparing the results against criteria established by recognized organizations such as RICOS and FSCB. This comprehensive approach equips the laboratory with vital insights into the capabilities and limitations of its analytical methods, ensuring they meet the standards required to deliver reliable analytical results and clinically meaningful interpretations [1, 2].

1.1. Reminder on C4 complement

All components of the complement system are acute-phase proteins synthesized during inflammatory episodes, with their levels rising rapidly in response. Conversely, various autoimmune diseases can lead to significant increases in the catabolism of complement proteins. Since complement component measurements reflect a static concentration resulting from the dynamic equilibrium between synthesis and catabolism, serial quantitative determinations are more clinically informative.

The complement system contributes to inflammation or tissue damage during immune responses and plays a crucial role in the pathogenesis of certain diseases. In such cases, complement activation is often triggered by an "abnormal" antibody (autoantibody), an immune complex, or a foreign body.

Elevated C4 levels are associated with acute-phase reactions and certain malignancies. Conversely, decreased C4 levels are observed in individuals with hereditary deficiencies or immunological disorders that lead to increased complement consumption. Reduced C4 concentrations can be seen in conditions such as hereditary or acquired angioedema, complement activation due to immune complex diseases, decreased synthesis in liver diseases, or increased consumption in the context of glomerulonephritis, systemic lupus erythematosus (SLE), rheumatoid arthritis, respiratory distress syndrome, autoimmune hemolytic anemia, cryoglobulinemia, or sepsis[5].

Total hereditary C4 deficiency is rare, whereas partial C4 deficiency is more common. Both partial and complete hereditary C4 deficiencies are associated with immune complex diseases, SLE, autoimmune thyroiditis, and juvenile dermatomyositis. Infections linked to C4 deficiency include bacterial or viral meningitis[3, 4]

2. Material and methods

The C4 assay is an immunoturbidimetric test that measures the increase in sample turbidity caused by the formation of insoluble immune complexes upon the addition of anti-C4 antibodies to the sample. The sample containing complement C4 is incubated with a buffer (R1), and the sample blank is determined before the addition of the anti-C4 antibody (R2). In the presence of an excess of the appropriate antibody, the C4 concentration can be measured based on the turbidity.

This prospective study was conducted over a 30-day period in the biochemistry laboratory of the Mohammed VI University Hospital. The study was divided into two phases. The first phase aimed to assess reproducibility, also referred to as intermediate precision, by running daily internal controls across three measurement levels: low, medium, and high, over the 30-day period to evaluate consistency. During this phase, a selection of serum samples with C4 complement levels evenly distributed across the measurement range was made. These levels were then categorized into three groups: low, medium, and high.

In the second phase, repeatability was evaluated by performing thirty consecutive measurements for each sample. The analytical procedure was carried out using the Alinity ci C4 complement reagent kit on the ABBOT Alinity C system. The study adhered to an operational approach based on the recommendations outlined in the COFRAC GTA 04 accreditation technical guide. Statistical analysis of the results was conducted using the EVM intermediate module provided by BYG Informatics, serving as an intermediary software bridging the gap between the Alinity platform and the iLab result validation software.

3. Results

3.1. Reproducibility results

Intra-laboratory reproducibility, also referred to as **intermediate fidelity**, is evaluated by conducting repeated measurements of samples under varying operational conditions, such as different time points, reagent batches, calibration settings, operators, and equipment. This methodology allows for the assessment of the impact of these variables on the test results. The data obtained from these measurements are subsequently analyzed to calculate the mean, standard deviation, and coefficient of variation (CV%) across different series, as well as within-series, between-series, and for the entire dataset [6].

The intermediate fidelity results were deemed acceptable at all three levels—low, medium, and high—yielding coefficients of variation (CV) of 4.5%, 4.22%, and 3.13%, respectively. The CV for reproducibility at each level met the predefined criteria, remaining within the acceptable limits established by both the SFBC (quality control system) and the RICOS (global quality control network). To facilitate a clearer interpretation of these results, graphical representations using Levey-Jennings plots are provided (Fig. 1, Fig. 2, and Fig. 3).

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

Level of IQC	Numbers of value	Mean (g/l)	Standard deviation en g/l	Coefficient variation CV(%)	Reference CV: FSBC 1999	References CV: RICOS (%)
Low	30	0.16 g/l	0.007g/l	4.5%	8%	4.45%
Medium	30	0.24 g/l	0.010 g/l	4.22%	6%	4.45%
High	30	0.35 g/l	0.011 mg/l	3.13%	5%	4.45%

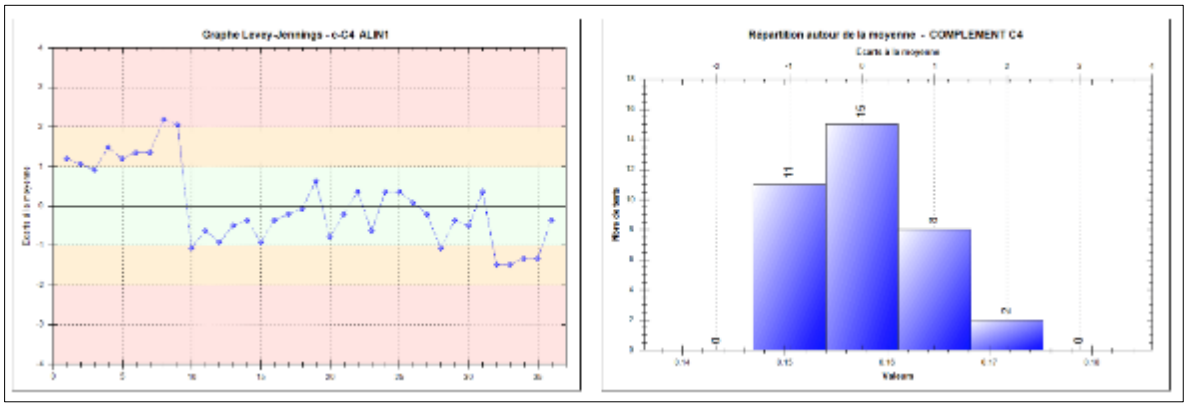

Figure 1 Low level of intermediate fidelity: Levey Jennings graph and the distribution around the mean

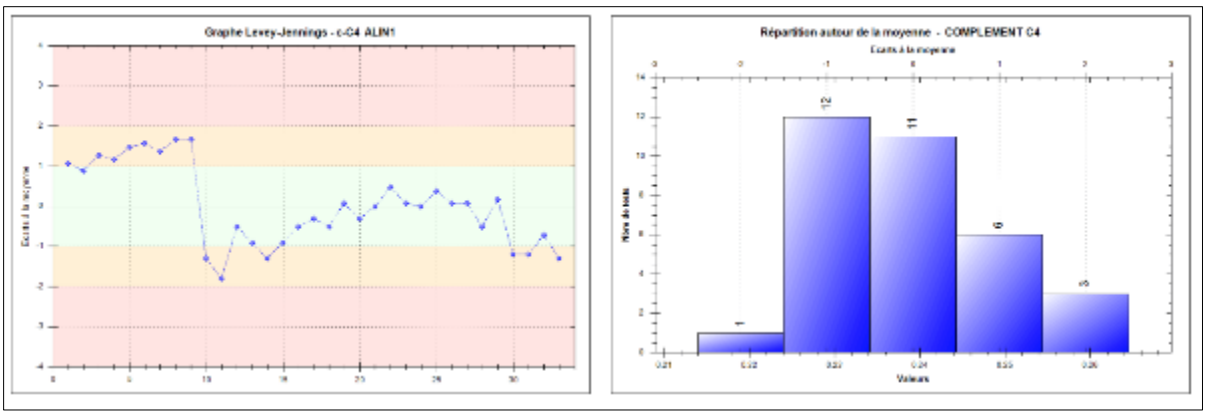

Figure 2 Medium level of intermediate fidelity: Levey Jennings graph and the distribution around the mean

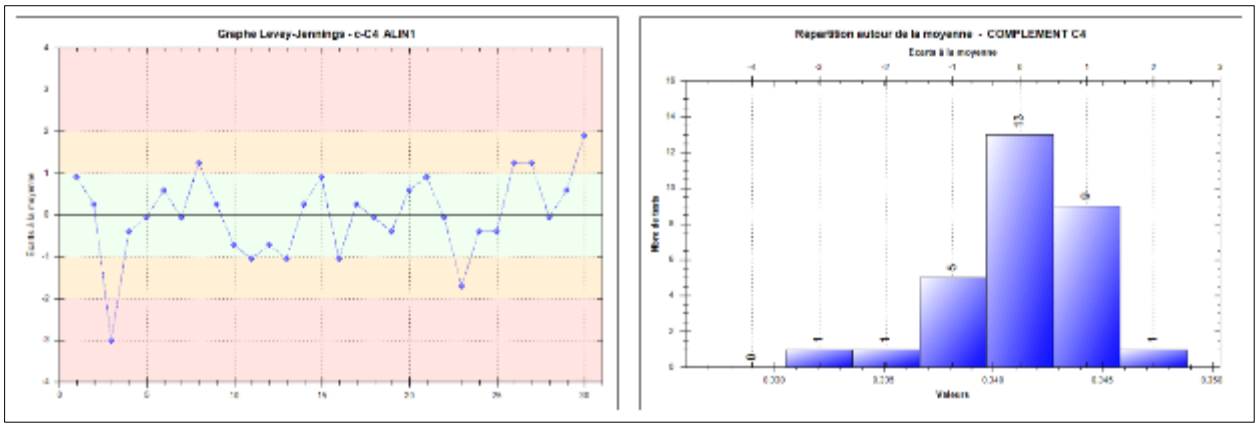

Figure 3 High level of intermediate fidelity: Levey Jennings graph and the distribution around the mean

3.2. Repeatability results

The findings of this study demonstrated excellent repeatability across all concentration levels—low, medium, and high—as reflected by the coefficients of variation (CVs): 0.89% for CV1, 1.29% for CV2, and 0.90% for CV3 (Table 2).These results are further detailed and effectively visualized using Levey-Jennings plots, providing a comprehensive representation of the data (Figures 4, 5, and 6).

Table 2 Repeatability results of blood assay by level with comparison to FSBC and RICOS data

Level of IQC	Numbers of value	Mean (g/l)	Standard deviation en g/l	Coefficient variation CV(%)	Reference CV: FSBC 1999	References CV: RICOS (%)
Low	30	0.15 g/l	0.001g/l	0.89%	7.27%	4.04%
Medium	30	0.24 g/l	0.003 g/l	1.29%	5.45%	4.04%
High	30	0.34 g/l	0.003 mg/l	0.90%	4.54%	4.04%

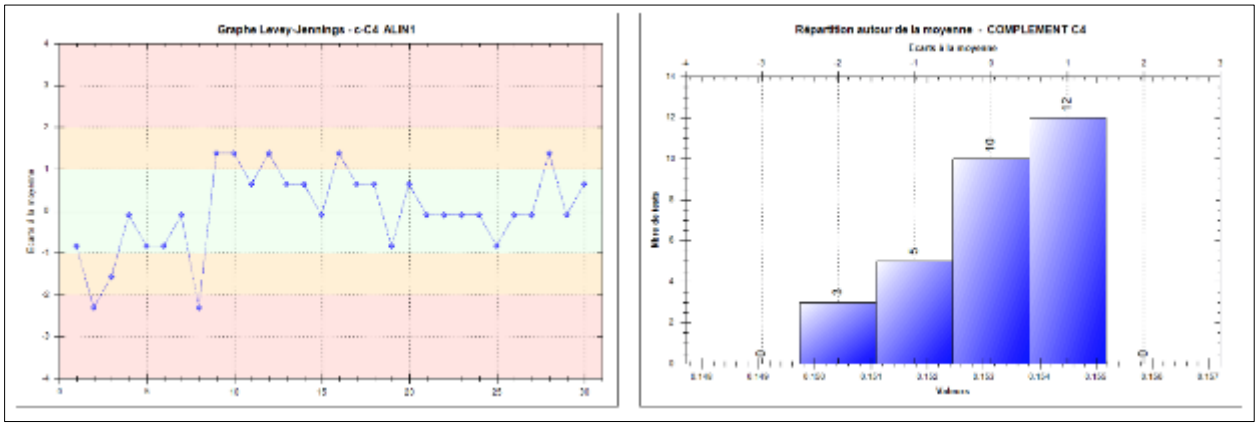

Figure 4 Low Level of Repeatability: Levey Jennings graph and the distribution around the mean

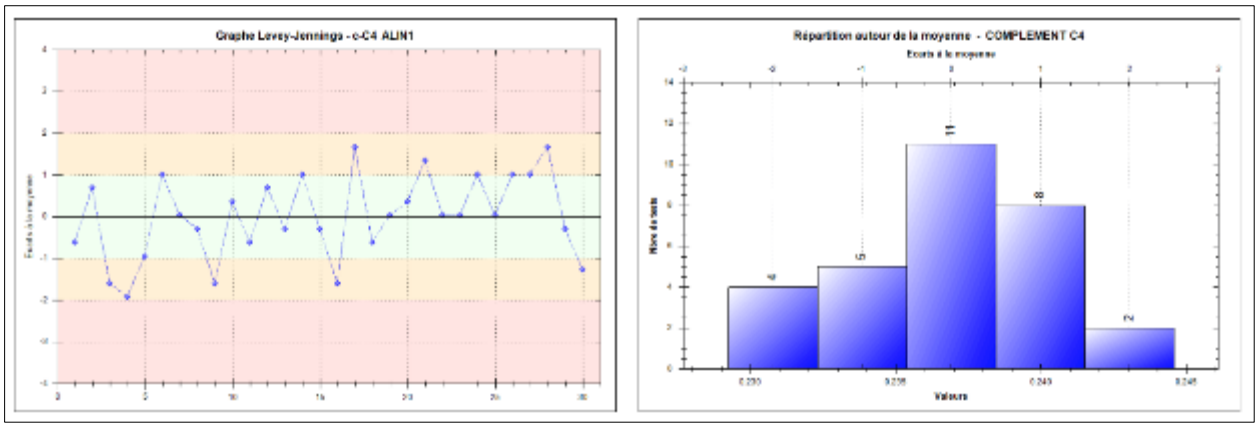

Figure 5 Medium Level of Repeatability: Levey Jennings graph and the distribution around the mean

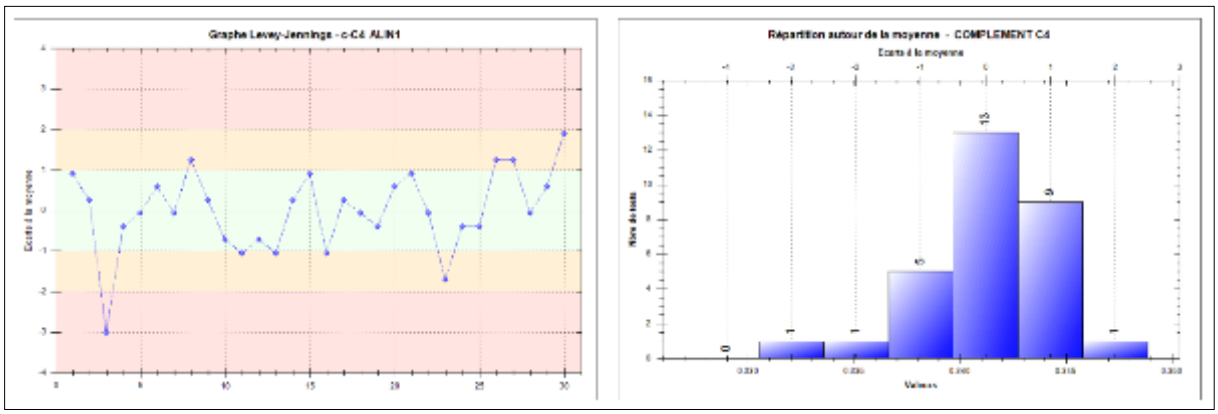

Figure 6 High Level of Repeatability: Levey Jennings graph and the distribution around the mean

4. Discussion

The activation of the complement system occurs through two main pathways: the classical pathway and the alternative pathway. The C4 fraction of the complement plays a critical role in activation via the classical pathway. A decrease in C4 levels is common, while a complete absence of C4 is rare. Reduced or absent C4 levels are observed in conditions such as immune complex diseases, systemic lupus erythematosus (SLE), autoimmune thyroiditis, and juvenile dermatomyositis.

The onset of SLE in patients with C4 deficiency can often be diagnosed early, and the disease progression tends to be less severe compared to patients with normal complement levels. Infections such as bacterial and viral meningitis, streptococcal and staphylococcal infections, and pneumonia are frequently associated with decreased C4 levels.

C4 measurement allows for better differentiation when C3 levels are also reduced. If C4 concentration is normal, there is a high probability that the alternative pathway has been activated. C4 testing is primarily used for monitoring conditions associated with hypocomplementemia.

As an acute-phase protein, C4 levels increase during inflammatory responses. Its concentration rises in systemic infectious diseases, chronic non-infectious inflammatory states (particularly in chronic forms of rheumatoid arthritis), and physiological conditions such as pregnancy. However, the increase rarely exceeds twice the normal range and can mask reductions caused by its consumption.

Ensuring proficiency in the laboratory methodologies employed by biologists is an ongoing priority. The verification and validation of these methods are mandated by both regulatory frameworks (e.g., the Moroccan Guide for the Good

Performance of Medical Laboratory Analyses) and normative standards (e.g., ISO 15189:2012) [6]. By setting predefined analytical objectives, such mastery facilitates the production of accurate and reliable results.

Reproducibility testing is a pivotal evaluation to determine the consistency of assay outcomes under varying conditions, such as differences in operators, time intervals, reagent batches, and calibration processes—factors that can influence result reliability. To measure this variability, the Coefficient of Variation (CV) is employed. The CV quantifies dispersion by expressing the extent of deviation from the mean as a percentage, providing a critical indicator of the precision and stability of the assay.

At low, medium, and high levels, the Coefficient of Variation (CV) values are 4.5%, 4.22%, and 3.13%, respectively. These relatively low CV values indicate that the assay consistently produces reliable results under varying conditions. The reproducibility analysis demonstrates that the immunoturbidimetric method exhibits robustness and stability, even when factors such as operator variability or reagent batch changes are introduced.

The low CV values confirm that the assay consistently yields results that remain close to the mean, ensuring high precision. This level of reliability is critical in medical testing, where consistent and reproducible outcomes are essential for informed clinical decision-making. Furthermore, the alignment of CV values with established quality control thresholds verifies that the assay adheres to industry standards for reproducibility, thereby validating its appropriateness for accurate and reliable diagnostic applications.

The repeatability test focuses on the precision of the assay under controlled and optimal conditions. This is important because it assesses the ability of the method to yield similar results when the same sample is analyzed multiple times. The CV values for repeatability are remarkably low: CV1 = 0.89%, CV2 = 1.29%, and CV3 = 0.90%. These values indicate an extremely small amount of variability, reaffirming the high precision of the assay. The exceptionally low CV values emphasize that the assay's outcomes are extremely stable and predictable under controlled conditions. This level of precision is essential

in clinical testing, where small variations can have significant implications for patient care. The alignment of the CV values with quality control standards underscores the assay's reliability and suitability for generating repeatable results. The assay demonstrates low variability and high precision across

varying conditions and repeated analyses of the same sample. These qualities are paramount in clinical diagnostics, where accurate and dependable results are crucial for patient care. The comparison to quality control standards provides an objective validation of the assay's performance, reassuring researchers and healthcare professionals that the method produces consistent and trustworthy results. The meticulous evaluation of variability ensures that the assay meets industry standards and can be confidently employed in clinical decision-making processes.

5. Conclusion

In conclusion, the verification of the analytical performance of the C4 complement assay on the Abbott Alinity CI analyzer using the immunoturbidimetric method demonstrated robustness, reliability, and precision. The results for reproducibility and repeatability are exceptional, conforming to the standards established.

by RICOS and the guidelines in the Valtec protocol (FSCB). These attributes are essential in clinical diagnostics, where the accuracy and reliability of results are critical for patient care. 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.

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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