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(RESEARCH ARTICLE)

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Verification of analytical performance of Complement C3 on the Abbott Alinity ci®: Experience of the central laboratory of Mohammed VI University Hospital of Oujda

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Abstract

The verification of analytical methods is a requirement outlined by the International Organization for Standardization (ISO). This process involves evaluating the performance of an analytical method according to a well-defined protocol and subsequently comparing it with pre-established analytical objectives.

In our study, we conducted an evaluation of the analytical performance of Complement C3 using the Abbott kit on the Alinity CI analyzer in the biochemistry laboratory of Mohammed VI University Hospital in Oujda. The methodology employed adheres to the recommendations of the French accreditation committee (COFRAC) accreditation technical guide SH GTA 04, focusing on the assessment of reproducibility and repeatability.The results obtained demonstrate good repeatability, with CV1 = 1.04%, CV2 = 1.13%, and CV3 = 0.97% for levels 1, 2, and 3, respectively. Intra-laboratory reproducibility was also satisfactory for levels 1, 2, and 3, with CV1 = 4.8%, CV2 = 3.13%, and CV3 = 2.9%. Confirming the performance of this method is essential, given the qualitative and quantitative importance of Complement C3 measurement in our daily practice. It's important to note that the accuracy and reliability of examination results are influenced not only by laboratory personnel, equipment, and environmental conditions, but also by the methods utilized and their eventual validation or verification.

Keywords: Complement C3; Verification; Repeatability; Reproducibility; Alinity CI

1. Introduction

Quality is a continuous process designed to ensure the consistent and accurate performance of tests. A quality assurance system includes all internal and external laboratory activities, integrating proper laboratory practices and enhanced management skills. Its objective is to ensure that every test conducted by the laboratory is both accurate and reliable. The adoption of quality principles in medical laboratories requires the establishment of a focused quality management process to maintain the reliability of the results[1]. Significant efforts have been made in recent years to enhance quality within clinical laboratories, particularly through the implementation of accreditation based on ISO 15189 standards, which address both the technical and managerial competencies of a laboratory. The accreditation process encompasses the validation, verification, and quality assurance of methods.[2]

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.

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C3 is a central component of the complement system. The activation of the classical, lectin, and alternative pathways leads to the cleavage of C3, producing C3b and the anaphylatoxin C3a. Upon the generation of C3b, the thioester bond is cleaved, allowing this highly reactive molecule to covalently bind to target surfaces. The interaction of the zymogen factor B with C3b, followed by its cleavage by factor D, results in the formation of the alternative pathway C3 convertase, C3bBb. This sequence of reactions constitutes an amplification loop[3].

In this study, we conducted a method verification protocol for complement C3 using Abbott's Alinity CI® automated system. The objective of this work is to establish a foundational study that supports the accreditation process and aligns with the quality assurance efforts to which our laboratory is fully dedicated.

1.1. Reminder on C3 complement

The complement system comprises a group of at least 20 circulating plasma proteins and several protein receptors that interact in a regulated proteolytic cascade to eliminate invasive bacteria and prevent immune complex deposition. Complement activation results in decreased concentrations of C3 and/or C4 due to the consumption of intact proteins.The complement cascade can be activated through two distinct pathways. The classical pathway is triggered by immune complexes or antibodies bound to bacteria and viruses(Figure 1). It begins with the binding of the C1q subunit of C1 to the Fc fragment of the antibody, followed by the proteolytic activation of C3 via C4 cleavage. Independently of antibodies, the alternative pathway is activated by microorganisms, polysaccharides, spontaneous autolysis of C3, or aggregated immunoglobulins. Unlike the classical pathway, the alternative pathway does not require C4.Since C3 is a common component of both pathways, reduced C3 levels indicate overall complement activation. Decreased C3 concentrations are observed in inflammatory and infectious diseases, particularly in glomerulonephritis and systemic lupus erythematosus (SLE). Depending on the pathway activated, C4 levels may be decreased or remain normal. Isolated reductions in C4 without concurrent C3 depletion occur in cases of hereditary or acquired angioedema.Genetic deficiencies of both complement factors have been reported. Both C3 and C4 act as acute-phase proteins, and their elevation during inflammatory processes can obscure mildly accelerated complement consumption[4].

Complement C3 is a critical component of the immune system, functioning alongside other factors to defend the body against pathogenic invasion. Upon activation through the classical or alternative pathways, the complement system targets biological membranes, potentially leading to cell lysis. The complement cascade in humans comprises a series of distinct plasma proteins. Measuring C3 levels is essential for detecting both congenital and acquired deficiencies. Conversely, elevated C3 levels are often observed in a range of inflammatory and necrotic conditions, reflecting its role in the acute-phase response of plasma proteins[5].

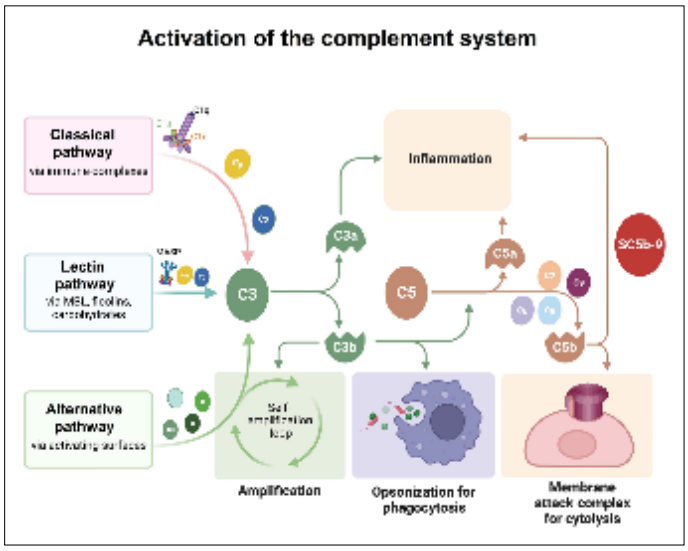

Figure 1 Schematic representation of the three activation pathways of the complement system

2. Materials and methods

The C3 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-C3 antibodies to the sample. The sample containing C3 is

incubated with a buffer (R1), and the sample blank is determined before the addition of the anti-C3 antibody (R2). In the presence of an excess of the appropriate antibody, the C3 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 C3 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 C3 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 known as intermediate fidelity, is assessed by performing repeated measurements of samples under varying operational conditions, including different times, reagent batches, calibrations, operators, and equipment. This approach evaluates the influence of these factors on the test results. The collected data is then analyzed to compute the mean, standard deviation, and coefficient of variation (CV%) for each series, within-series, between-series, and for the entire dataset [6].

The results of intermediate fidelity were deemed acceptable for all three levels—low, medium, and high—with coefficients of variation (CV) of 1.04%, 1.13%, and 0.97%, respectively. The reproducibility CV for each level was satisfactory, remaining within the established limits defined by both the SFBC (quality control system) and the RICOS (global quality control network). To provide a clearer understanding of the findings, the results are presented graphically using Levey-Jennings plots (Fig. 2, Fig. 3, and Fig. 4).

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	0.91 g/l	0.043 g/l	4.8%	8%	2.6%
Medium	30	1.39 g/l	0.044 g/l	3.13%	6%	2.6%
High	30	2.01 g/l	0.058 g/l	2.9%	5%	2.6%

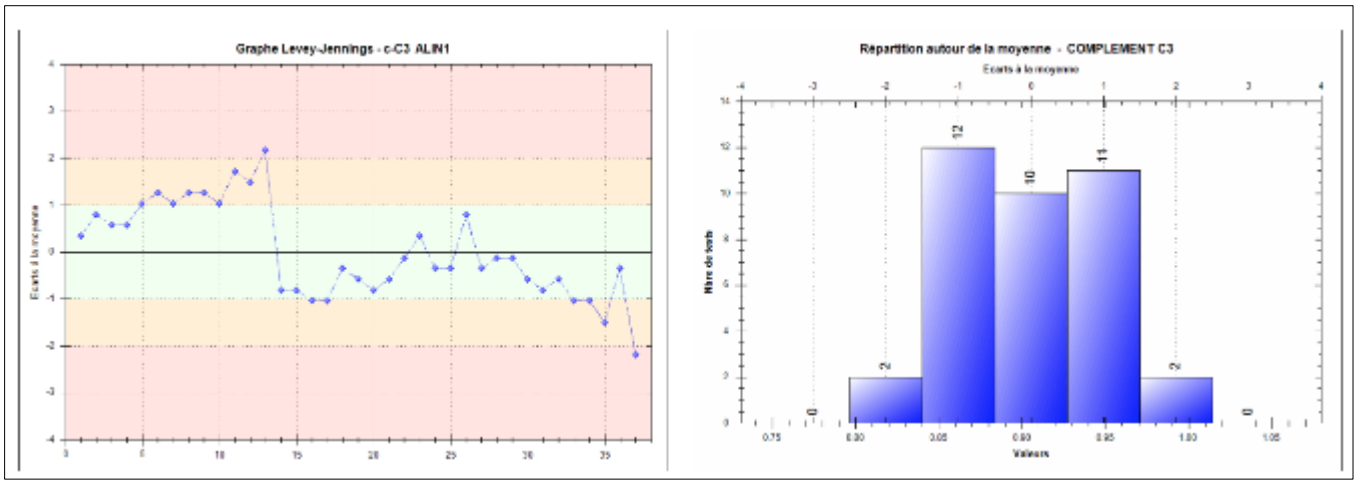

Figure 2 Low level of intermediate fidelity: Levey Jennings graph and the distribution around the mean

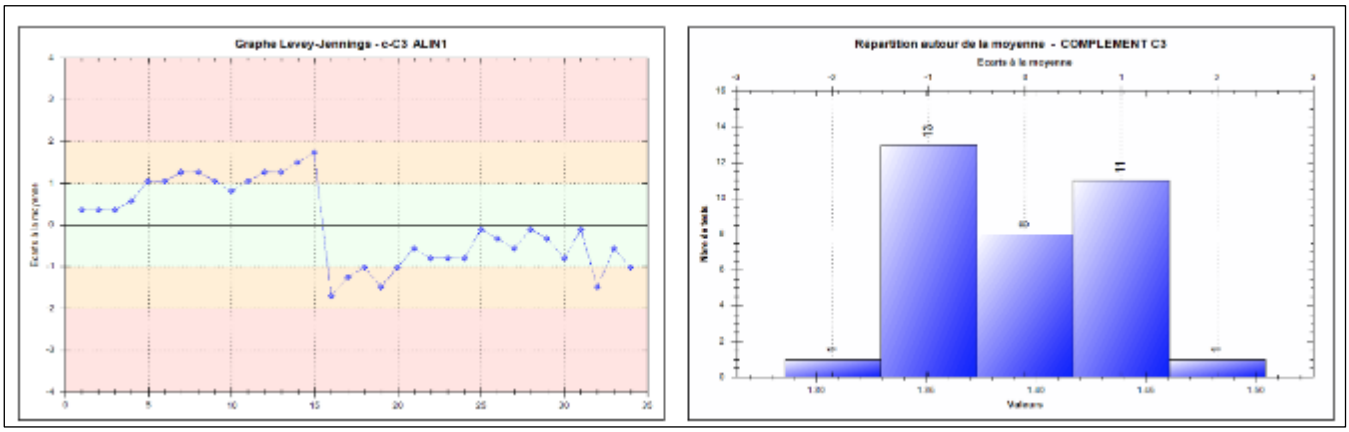

Figure 3 Medium level of intermediate fidelity: Levey Jennings graph and the distribution around the mean

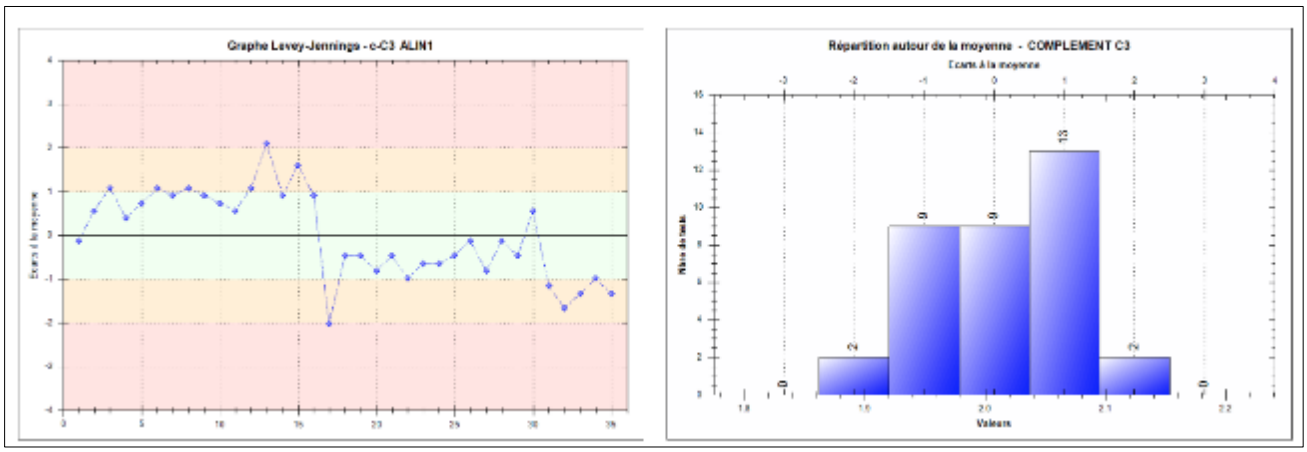

Figure 4 High level of intermediate fidelity: Levey Jennings graph and the distribution around the mean

3.2. Repeatability Results:

Repeatability is assessed through repeated testing of the same samples by the same operator under consistent conditions, accounting for all measurement factors such as reagents, calibration, instrument, and operator, and within the shortest possible time frame. This test allows the initial performance to be determined and the proper functioning of the system (instrument/reagent) for the target analyte to be verified. Variability is then measured using coefficient of variation (CV) values. As shown in Table 2, the results for the various complement C3 assay verification criteria

demonstrate satisfactory repeatability across all three levels (low, medium, and high), with CVs of 1.04%, 1.13%, and 0.97%, respectively, from 30 samples.

Table 2 Repeatability results for C3 complement on the Alinity i® automated system by level with comparison to SFBC 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	0.82 g/l	0.009g/l	1.04%	6%	1.95%
Medium	30	1.32 g/l	0.015 g/l	1.13%	4.5%	1.95%
High	30	1.92 g/l	0.019g/l	0.97%	3.75%	1.95%

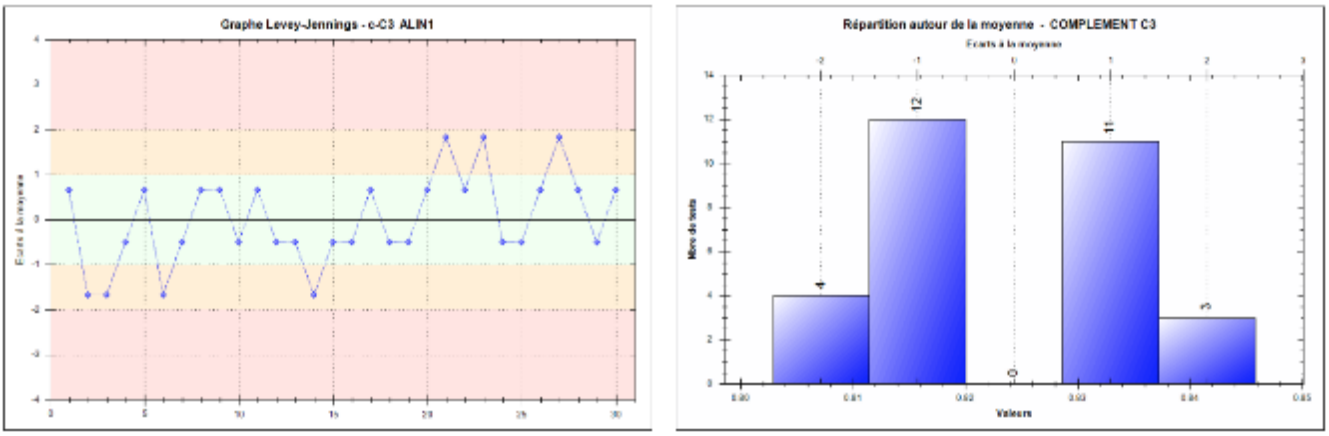

Figure 5 Low Level of Repeatability: Levey Jennings graph and the distribution around the mean – C3 complement

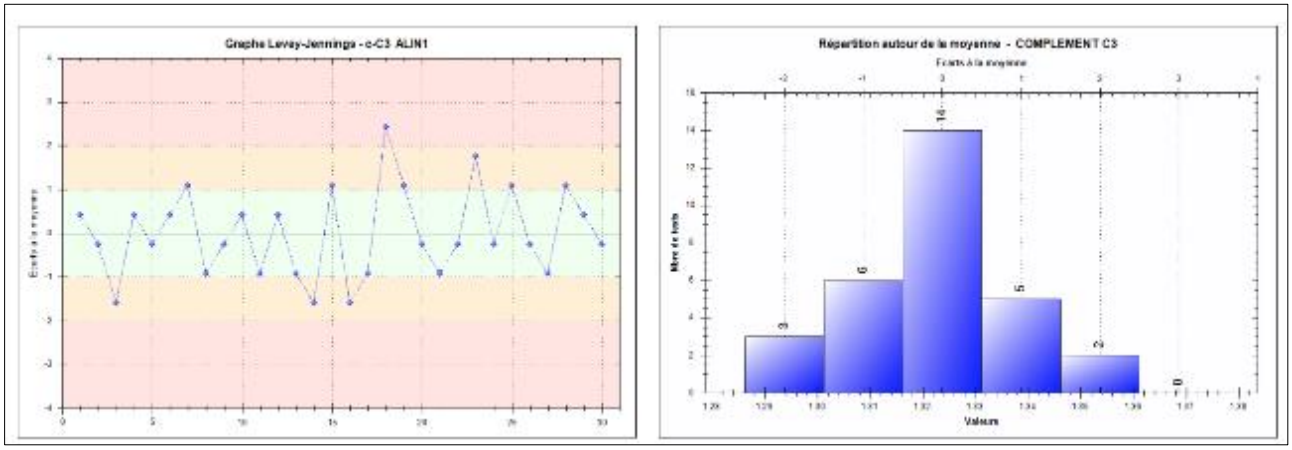

Figure 6 Medium Level of Repeatability: Levey Jennings graph and the distribution around the mean – C3 complement

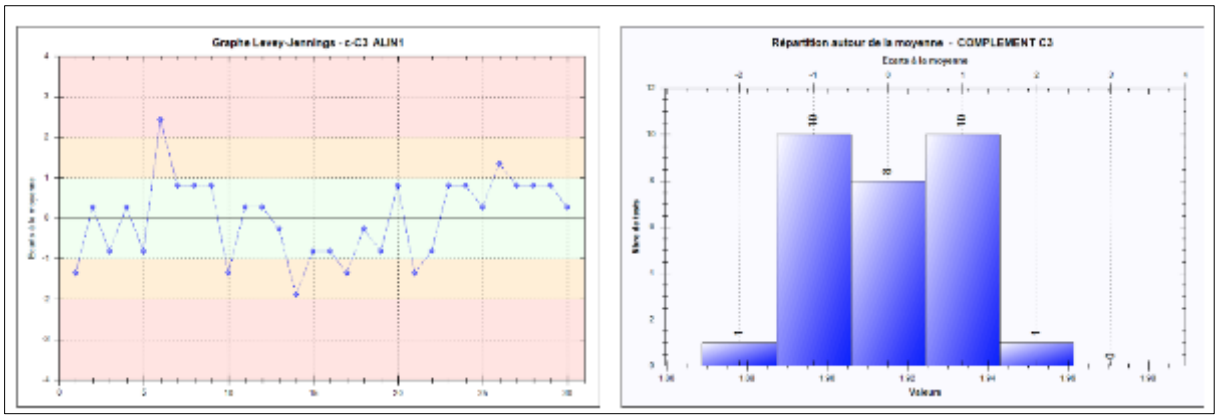

Figure 7 High Level of repeatability: Levey Jennings graph and the distribution around the mean – C3 complement

4. Discussion

C3 represents a pivotal component of the complement system. Its activation through any of the three principal pathways—the classical, lectin, or alternative pathways—triggers the terminal complement pathway and facilitates the release of the anaphylatoxin C3a. Both the activation of the terminal pathway and signaling mediated by C3a and its inactivated form, C3a-desArg, via the C3a receptor and the C5a-like receptor 2, respectively, are capable of inducing inflammatory, immunomodulatory, and metabolic responses.

C3 has been closely associated with various metabolic disorders, including adiposity, dyslipidemia, insulin resistance, liver dysfunction, and diabetes. It is increasingly acknowledged as a cardiometabolic risk factor and is hypothesized to contribute to both macrovascular and microvascular complications in diabetes. Additionally, C3's interactions with the coagulation system may promote a procoagulant, hypofibrinolytic, and ultimately prothrombotic state. Emerging evidence suggests that in diabetes, C3 becomes incorporated into fibrin clots, altering clot structure and function.

Collectively, epidemiological and experimental findings strongly support the involvement of C3 in metabolic, atherosclerotic/atherothrombotic, and microangiopathic processes. These observations underscore the need for further research to clarify the roles of complement activation and regulation in cardiometabolic disorders[7]. C3 is now recognized as a component involved in the specific mechanisms of adaptive immunity.

The Abbott Alinity ci is a multiparametric system designed to integrate clinical chemistry and immunoassay testing, facilitating the quantification of a broad array of standard biochemical markers and specific proteins.

In this study, we conducted an analytical performance verification of the C3 complement assay using the chemistry module of the Abbott Alinity ci analyzer. This procedure falls under the "flexible category A" for method verification, a process that can be carried out in accordance with the COFRAC guide SH-GTA-04(10). A full method validation was not required; instead, method verification, in alignment with laboratory practices, is deemed sufficient. The sensitivity and specificity of the techniques were not necessary to verify. Additionally, the central laboratory of Mohammed VI University Hospital in Oujda is dedicated to upholding the highest standards of analytical performance, ensuring the delivery of reliable and accurate laboratory results for patients.

This verification is crucial, as it complies with both regulatory standards (as outlined in the Moroccan Guide for the Proper Execution of Medical Laboratory Analyses, GBEA) and normative requirements (ISO 15189:2022). By establishing predetermined analytical objectives through this process, it ensures the generation of accurate and reliable results.

The intermediate fidelity test enabled the evaluation of assay result consistency in the presence of various influencing factors. These factors include variations in operators, time intervals, reagent kits, and calibrations, all of which can affect result reliability. To quantify this variability, coefficients of variation (CV) were employed . The coefficients of variation obtained from the intermediate fidelity study demonstrated the reliability of the C3 complement assay. The results at all three levels were generally satisfactory (Table 1), with CV1, CV2 values of 1.04%, 1.13%, and 0.97% respectively. The low CV values suggest that, even with changes in variables such as operator or reagent lot, the assay consistently produces results that are closely aligned with the mean. This reliability is crucial in medical diagnostics, where

consistent and accurate test results are essential for informed clinical decision-making. Furthermore, the alignment of the CV values with established quality control limits confirms that the assay meets the supplier's reproducibility standards, further validating its suitability for precise diagnostic applications.

The repeatability test primarily evaluates the precision of the assay under controlled and optimal conditions. This assessment is critical as it determines the method's ability to generate consistent results when the same sample is analyzed multiple times.

In examining the repeatability across three levels (low, medium, and high), 30 values were analyzed for each level, revealing remarkably low coefficients of variation (CV): CV1 = 1.04%, CV2 = 1.13%, and CV3 = 0.97%. These values indicate a low degree of variability, highlighting the high precision of the assay.

The exceptionally low CV values underscore the assay's high stability and predictability when conducted under controlled conditions. This level of precision is critical in clinical testing, where even small variations can have substantial implications for patient care.

The central laboratory of Mohammed VI University Hospital in Oujda has adopted a quality strategy that includes a method verification protocol. This approach will facilitate the establishment of a reliable accreditation process for the analyses conducted in our laboratory. As a key reference center in the Eastern region of Morocco, our laboratory not only addresses the diagnostic needs of referred and hospitalized patients but also plays a crucial role in evaluating the health status of the general population through various scientific studies[8].

5. Conclusion

In conclusion, the verification of the analytical performance of the C3 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.

References

[1] Ségula J-P, Hermès I, Iché J-M, Lartigau J, Mura P. Guide de bonne exécution des analyses de biologie médicale (GBEA): son application dans le secteur hospitalier public. n.d.

[2] Pum J. A practical guide to validation and verification of analytical methods in the clinical laboratory. Adv Clin Chem, vol. 90, Academic Press Inc.; 2019, p. 215–81. https://doi.org/10.1016/bs.acc.2019.01.006.

[3] Frémeaux-Bacchi V, Miller EC, Kathryn Liszewski M, Strain L, Blouin J, Brown AL, et al. Brief report Mutations in complement C3 predispose to development of atypical hemolytic uremic syndrome. 2008. https://doi.org/10.1182/blood-2008-01.

[4] Complement C3c FS* (Complement C3c FS*). 1802.

[5] COMPLEMENT C3 (C3). n.d.

[6] Medicale EB. Section Santé humaine GUIDE TECHNIQUE D'ACCREDITATION DE VERIFICATION (PORTEE A) / VALIDATION (PORTEE B) DES METHODES. n.d.

[7] Hertle E, Van Greevenbroek MMJ, Stehouwer CDA. Complement C3: An emerging risk factor in cardiometabolic disease. Diabetologia 2012;55:881–4. https://doi.org/10.1007/s00125-012-2462-z.

[8] Sebbar EH, Sam H, Sidqi Z, Saalaoui E, Choukri M. Calcium intakes in the diet of eastern Morocco's population. Journal of Pharmacy and Nutrition Sciences 2018;8:91–6. https://doi.org/10.6000/1927-5951.2018.08.03.2.