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Verification of the analytical performance of the serum Folate assay on the Abbott Alinity ci®

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Abstract

In the evolving landscape of clinical diagnostics, the significance of folate takes center stage as precision and innovation shape advancements in analytical methodologies. Folate, a pivotal element in cellular metabolism, governs physiological processes and serves as an indicator for various medical conditions. This study employs the Chemiluminescent Microparticle Immunoassay (CMIA), known for its accuracy, to assess the reproducibility and repeatability of serum folate levels.

Folate's role in cellular metabolism influences diverse physiological functions, making its precise quantification crucial for identifying and monitoring conditions such as folate deficiency. The CMIA method emerges as a robust approach, leveraging immunological specificity for high precision and reliability.

Utilizing the Abbott Alinity ci® Analyzer, a technologically advanced clinical chemistry instrument, this study incorporates a systematic analytical method verification procedure. This involves quantification through a standardized protocol and a comparative analysis against criteria set by esteemed societies (RICOS and FSCB), ensuring comprehensive insights into analysis techniques.

The reproducibility test, evaluating the impact of various factors on assay results, reveals low Coefficient of Variation (CV) values (CV1: 10.25%, CV2: 8.58%, CV3: 9.13%) across different levels. The results align with quality control limits, emphasizing the method's reliability. Repeatability assessment demonstrates exceptionally low CV values (CV1: 4.84%, CV2: 3.41%, CV3: 1.89%), highlighting the method's stability and precision under controlled conditions.

Keywords: Serum Folate assay; Abbott Alinity ci analyzer; precision; Reliability; Biochemistry laboratory; Mohammed VI University Hospital; Reproducibility assessment; Repeatability testing; Chemiluminescent microparticle immunoassay method; CMIA

1. Introduction

In the realm of clinical diagnostics, the spotlight now shifts to the vital role played by folate, as precision and innovation continue to shape advancements in analytical methodologies. Folate, a crucial element in cellular metabolism, not only governs various physiological processes but also serves as a sentinel for multiple medical conditions. As diagnostic platforms progress to meet escalating demands, a meticulous examination of the analytical capabilities of assays becomes essential, particularly when dealing with pivotal analytes like folate. This demands the utilization of methods renowned for accuracy, such as the Chemiluminescent Microparticle Immunoassay (CMIA).

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Folate, a central player in cellular metabolism, orchestrates a myriad of physiological functions, from contributing to energy provision to influencing signaling cascades crucial for cellular activities [1]. Its precise quantification sheds light on the complexities of metabolic pathways and facilitates the identification and monitoring of conditions like folate deficiency and other metabolic disorders. In this context, the Chemiluminescent Microparticle Immunoassay emerges as a robust approach [2], leveraging immunological specificity to deliver results characterized by high precision and reliability.

The Abbott Alinity ci® Analyzer stands as a hallmark of technological advancement in clinical chemistry instrumentation, promising enhanced throughput, efficiency, and accuracy. In the pursuit of data-driven diagnostics. Analytical method verification entails a systematic procedure that includes quantification through a standardized operational protocol, followed by a comparative analysis against criteria established by esteemed societies (such as RICOS and FSCB). This methodological evaluation equips laboratories with comprehensive insights into their analysis techniques, performance metrics, and constraints. Ensuring the dependability of analytical outcomes remains of paramount importance, fostering clinically relevant interpretations for both patients and healthcare providers.

1.1. Principal of the assay method:

This assay employs a two-step immunological process for the quantitative determination of folates in human serum, plasma, and erythrocytes (RBC) using the Chemiluminescent microparticle immunoassay (CMIA). Two pre-treatment steps are implemented to release folates from endogenous folate-binding proteins.

The sample and pre-treatment reagent 2 (dithiothreitol or DTT) are combined. Pre-treatment reagent 1 (potassium hydroxide or KOH) is added to an aliquot of the sample/reagent 2 mixture. An aliquot of the pre-treated sample, microparticles paramagnetically coated with folate-binding proteins (FBP: Folate Binding Protein), and the specific diluent for the assay are combined and incubated. Folates present in the sample bind to the FBP-coated microparticles. After washing, the conjugate of acide pteroic marked with acridinium is added to form a reaction mixture, followed by incubation. After another washing cycle, pre-activation and activation solutions are added [3].

The resulting chemiluminescent reaction is measured in relative light units (RLU). There is an inverse relationship between the quantity of folates in the sample and the RLU detected by the optical system.

2. Material and Methods

This prospective study was conducted in the biochemistry laboratory of Mohammed VI University Hospital over a 30-day duration. The investigation focused on the reproducibility and repeatability of Folate dosage.

The study comprised two distinct phases. In the initial phase, daily control measurements were performed at three levels—low, medium, and high—over 30 days to assess reproducibility and ensure consistency. Subsequently, in the second phase, a collection of serum samples with varied Folate concentrations spanning the measurement spectrum was obtained. These samples were categorized into three groups based on their Folate levels—low, medium, and high. For each sample, 30 replicates were conducted to evaluate repeatability.

The analytical procedure involved the utilization of the Folate reagent kit on the chemistry module. Data manipulation was facilitated by the BYG middleware, acting as a bridge between the Alinity platform and the iLab result validation software. Coefficient of variation (CV) values obtained from our investigation were compared to standards set by respected professional bodies (FSCB and RICOS). The results of this analysis are presented in the subsequent sections.

3. Results

3.1. Reproducibility results

In the reproducibility test, the same sample undergoes analysis under varying conditions to assess the impact of factors such as operators, time, reagent batches, and calibrations on the results. The objective is to establish acceptance criteria for priors, particularly in decision support systems. The Coefficient of Variation (CV) is employed as a metric to quantify the variability of the results.

For the low, medium, and high levels, specific CV values were obtained (CV1: 10.25%, CV2: 8.58%, CV3: 9.13%). These results are visually represented on the Levey-Jennings graphs (Fig. 1, Fig. 2, Fig. 3).

The conclusion drawn for each level emphasizes that the CV of reproducibility is within acceptable limits and falls below the tolerated threshold. Furthermore, limits established by FSBC (a quality control system) and RICOS (a global quality control network) with expansion factors are considered. The obtained CV values are then compared against these predefined limits, as outlined in Table 1.

Table 1 Reproducibility results of the serum folate level with comparison to FSBC and RICOS data (with expansion coefficient k = 1.211)

Level of IQC	Number of values	Mean (ng/ml)	Standard Deviation	Coefficient of Variation CV (%)	Reference CV: FSBC 1999 (%)	Reference CV: RICOS (%)
Low	30	3.89	0.399	10.25	12.15	14.58
Medium	30	5.37	0.460	8.58	9.69	14.54
High	30	13.09	1.196	9.13	9.69	14.54

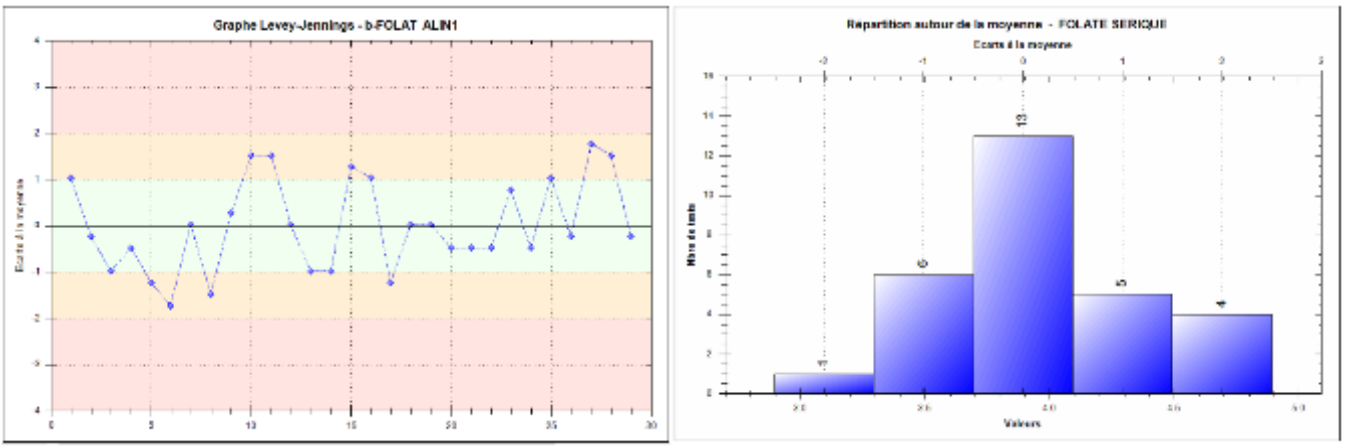

Figure 1 Low Level of Reproducibility: Levey Jennings graph and the distribution around the mean – Folate

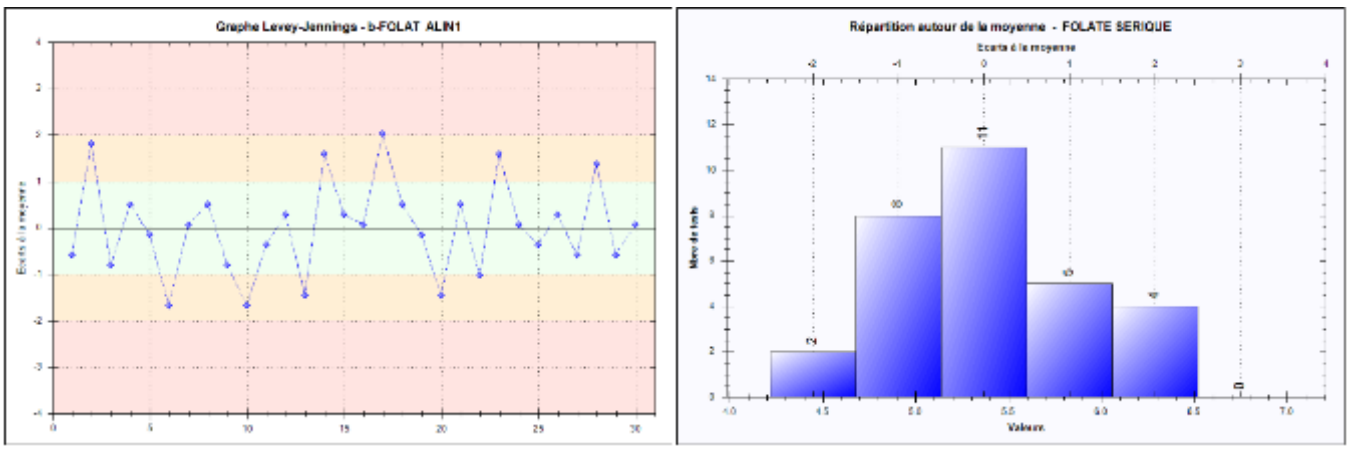

Figure 2 Medium Level of Reproducibility: Levey Jennings graph and the distribution around the mean – Folate

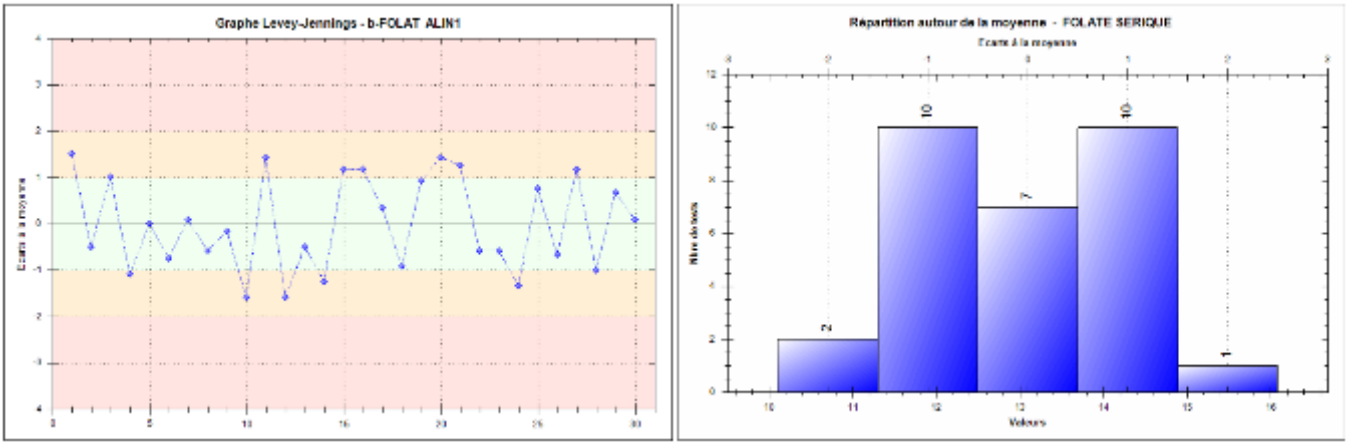

Figure 3 High Level of Reproducibility: Levey Jennings graph and the distribution around the mean – Folate

3.2. Repeatability results

The repeatability test entails examining the same sample under optimal conditions to evaluate the system's performance and functionality. Coefficient of Variation (CV) is employed to quantify variability.

For the low, medium, and high levels, the CV values are as follows: CV1 = 4.84%, CV2 = 3.41%, CV3 = 1.89%. These outcomes are depicted on the Levey-Jennings graphs (Fig. 4, Fig. 5, Fig. 6).

The conclusion for each level asserts that the CV of repeatability is accurate and remains below the tolerated limit. Similar to the intermediate fidelity results, the Free Statistical Benchmarking Consortium (FSBC) and RICOS limits with expansion factors are referenced. The CV values are then compared to these limits (Table. 2).

Table 2 Repeatability results of serum folate assay by level with comparison to FSBC and RICOS data (with expansion coefficient k = 1.211)

Level of IQC	Number of values	Mean (ng/ml)	Standard Deviation	Coefficient of Variation CV (%)	Reference CV: FSBC 1999 (%)	Reference CV: RICOS (%)
Low	30	4.97	0.241	4.84	9.09	10.90
Medium	30	9.37	0.320	3.41	7.27	10.90
High	30	17.22	0.326	1.89	7.27	10.90

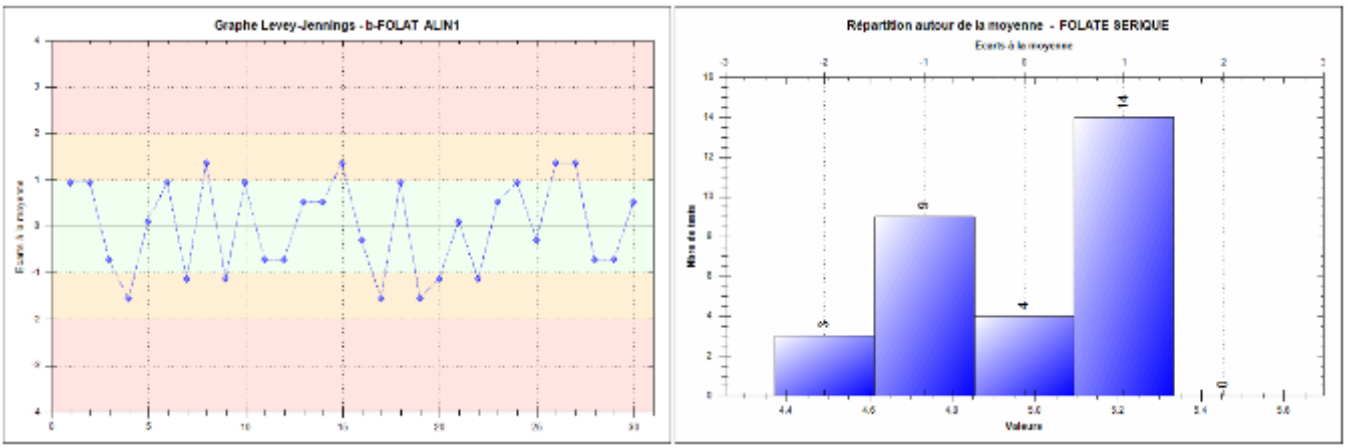

Figure 4 Low Level of Repeatability: Levey Jennings graph and the distribution around the mean – Folate

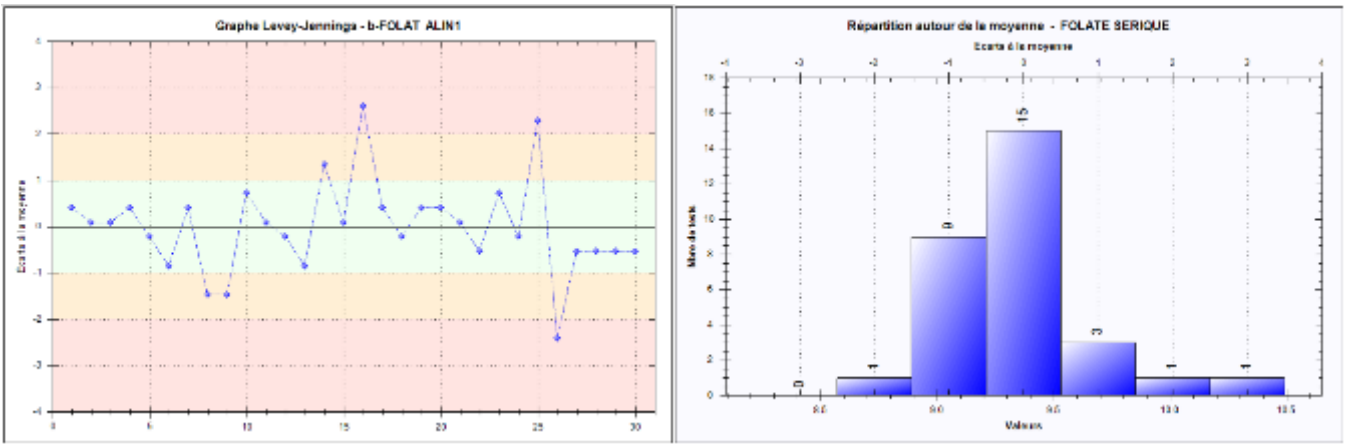

Figure 5 Medium Level of Repeatability: Levey Jennings graph and the distribution around the mean – Folate

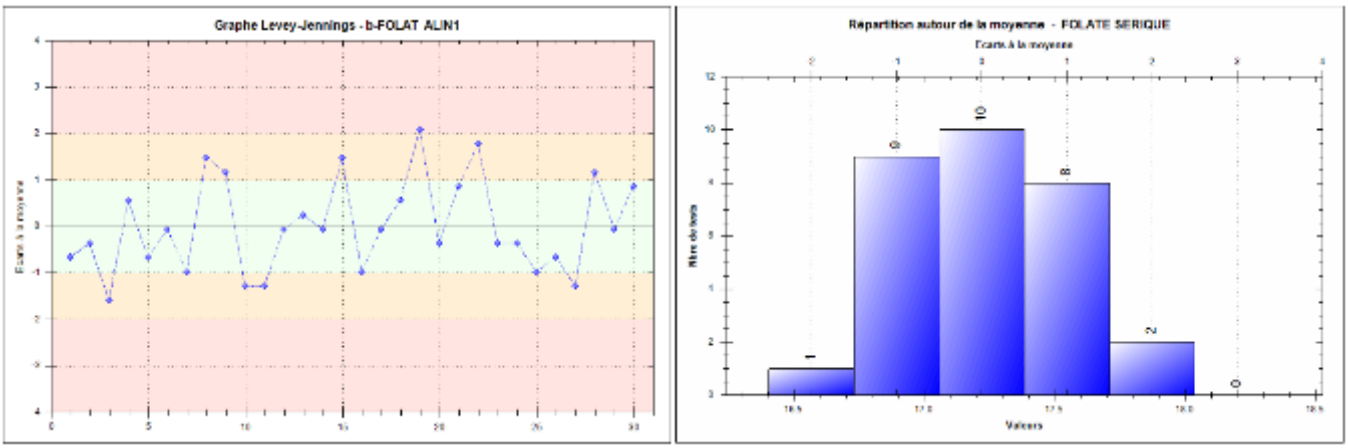

Figure 6 High Level of Repeatability: Levey Jennings graph and the distribution around the mean – Folate

4. Discussion

Folates are a class of vitamin-related compounds related to pteroylglutamic acid (PGA) that serve as cofactors during enzymatic transfer of monocarboxylic units in various metabolic pathways. The monocarboxylate metabolism, in which folates play a role, constitutes one of the most important biochemical reactions occurring in cells[4]. Folates are necessary for the synthesis of nucleic acids and mitochondrial proteins, amino acid metabolism, and other cellular processes involving monocarboxylic unit transfers. Folates can act as donors or acceptors of carbon. Since different metabolic pathways require carbon groups at different oxidation levels, cells contain numerous enzymes that modify the oxidation level of carbon groups transported by folates, leading to the formation of several forms of metabolically active folates. The predominant form of circulating folates is methyl-5-tetrahydrofolate (5-mTHF). A methyl group is transferred from 5-mTHF to cobalamin through the common metabolic pathway of folic acid and vitamin B12[5]. Folate deficiency can result from low dietary intake, malabsorption due to gastrointestinal diseases, inappropriate use due to enzymatic deficiency or antifolate treatment, substances such as alcohol or oral contraceptives, and increased folate needs, as during pregnancy.

As both vitamin B12 and folate deficiencies can lead to megaloblastic (macrocytic) anemia, proper treatment requires a differential diagnosis of the deficiency. Therefore, it is necessary to determine both the vitamin B12 level and the folate level. Low serum folate concentrations reflect the initial stage of negative folate balance and precede tissue depletion. Low erythrocyte folate concentrations reflect the second stage of negative folate balance and are more closely correlated with tissue levels and megaloblastic anemia.

Mastering the method employed by the biologist in the laboratory is a continual concern, and its verification/validation is both a regulatory requirement (Moroccan Guide for the Good Performance of Medical Laboratory Analyses) and a

normative one (ISO 15189:2022 standard) [6]. By establishing predetermined analytical objectives, this mastery allows for the generation of accurate and dependable results.

The reproducibility test serves as a critical assessment of how consistent the assay's results are when different variables are introduced. This includes variations in operators, time, reagent batches, and calibrations – all factors that can impact the reliability of the results. To quantify this variability, the Coefficient of Variation (CV) is used. The CV provides a percentage measure of how much the results deviate from the mean, indicating the level of dispersion or scatter in the data.

For each of the low, medium, and high levels of the reproducibility test the CV values are 10.25%, 8.58% and 9.13% respectively. These values are relatively low, which implies that the assay is producing consistent results across different conditions.

The reproducibility results suggest that the Chemiluminescent microparticle immunoassay method is robust and stable across various conditions. The low CV values indicate that even when different factors are altered, such as the operator or reagent batch, the assay consistently produces results that are close to the mean value. This reliability is crucial in medical testing, where consistency ensures that the test results can be trusted for clinical decisions. The fact that the CV values align with established quality control limits indicates that the assay meets industry standards for reproducibility, reinforcing its suitability for accurate diagnostic use.

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= 4.84%, CV2 = 3.41%, CV3 = 1.89%. These values indicate an extremely small amount of variability, reaffirming the high precision of the assay.

The repeatability results suggest that the Chemiluminescent microparticle immunoassay method provides consistent and highly precise measurements when analyzing the same sample multiple times. 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.

Both the reproducibility and repeatability results collectively reinforce the robustness and reliability of the serum folate assay using the Chemiluminescent microparticle immunoassay method. 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.

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[7], [8], [9], [10].

5. Conclusion

The study revealed compelling results in both the reproducibility and repeatability tests, showcasing the robustness and reliability of the CMIA method. Low Coefficient of Variation (CV) values obtained across different levels attest to the consistency and stability of the assay under varying conditions. The alignment of these results with quality control limits established by respected societies, including FSBC and RICOS, further underscores the method's adherence to industry standards.

In essence, the meticulous evaluation of variability and precision reaffirms the suitability of the CMIA method for serum folate assays in clinical settings. The outcomes of this study contribute valuable insights to the field of clinical

diagnostics, offering a robust and reliable approach for the quantification of serum folate levels, thereby enhancing the overall 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 declare no conflict of interest.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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