World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(RESEARCH ARTICLE)

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# Verification of methotrexate dosage method on Alinity c Abbott automated system in the biochemistry laboratory of CHU Mohammed VI d'Oujda

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World Journal of Biology Pharmacy and Health Sciences, 2024, 19(02), 261–267

Publication history: Received on 27 June 2024; revised on 09 August 2024; accepted on 12 August 2024

Article DOI: https://doi.org/10.30574/wjbphs.2024.19.2.0504

## Abstract

**Introduction:** This work describes the verification/validation procedure for methotrexate, an anti-metabolite from the antifolate group. Ensuring accurate and reliable measurements is a primary and ongoing concern for biologists in quality management. Based on the recommendations of the technical accreditation guide for human health, SH GTA of COFRAC according to ISO 15189, we performed the verification of a microparticle immunoassay method by chemiluminescence (CMIA) for the quantitative determination of methotrexate in human serum and plasma using the Architect system.

**Objectives:** The objective of this study was to verify the analytical method for methotrexate determination by assessing key criteria such as repeatability and reproducibility. This verification process aimed to ensure that the method meets the required standards and provides reliable and accurate results in clinical diagnostics.

**Results and Discussion:** The verification of criteria (repeatability and reproducibility) was conducted using routine patient samples from the CHU and internal and external quality controls. The data were processed using the Middleware EVM validation module.

The study of analytical performance demonstrated that repeatability conformed to the supplier's coefficients of variation (CV) and the data from learned societies (RICOS, SFBC). The results for intra-laboratory reproducibility were very satisfactory for level 1 with a CV of 3.34%, and fairly satisfactory for levels 2 and 3 with CVs of 4.74% and 4.57%, respectively.

**Conclusion:** In conclusion, the study showed that the results obtained for the different verification criteria of methotrexate measurement on our Alinity ci system are satisfactory when compared to the supplier's data and the standards of learned societies such as SFBC. These results confirm the analytical performance of the method.

**Keywords:** Method verification/validation; Repeatability; Reproducibility; Coefficient of variation

## 1. Introduction

Analytical method verification is the process of assessing the performance of an analytical method, quantifying it according to a standardized operating protocol, and then evaluating it against the standards determined by learned societies (RICOS, FSCB), thus providing the laboratory with a sound knowledge of its analytical methods, their performance and their limitations. In order to guarantee accurate analytical results and clinical interpretations

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beneficial to the patient and prescriber, the laboratory must ensure that these performances are sufficient [1,2]. In our study, we set out to evaluate the analytical performance of the methotrexate assay using an Abbott kit on the Alinity c automated system in the biochemistry laboratory of the CHU Mohammed VI d'Oujda. This work forms an essential basis for carrying out an accreditation procedure, and is a component of the quality approach to which the Biochemistry Laboratory of CHU Mohammed VI d'Oujda is committed [3].

### 1.1. Rappel

Methotrexate is an antimetabolite of the antifolate group. It is an analog of aminipterin, which is also derived from folic acid. The molecular structure of methotrexate differs from that of folic acid in that it has a hydroxyl group instead of amino group 4 on the pteridine ring, and no methyl group in the N position [4].

Methotrexate (MTX) is an antineoplastic agent marketed as tablets and injectable solutions for the treatment of placental choriocarcinoma, acute lymphoblastic leukemia in children and adults, osteosarcoma and other solid tumors (breast, bladder). Other non-cancer indications currently include autoimmune diseases such as rheumatoid arthritis, severe adult psoriasis, cortico-resistant sarcoidosis and ankylosing spondylitis [5].

This product belongs to the pharmacotherapeutic class of antimetabolics. It is a selective cytostatic of the S phase of cell replication, more active on rapidly proliferating cells (such as malignant or myeloid cells, thus inhibiting their growth and proliferation). It belongs to the chemical class of structural analogues of folic acid. Its action is exerted by inhibition of dihydrofolate reductase (DHFR) [formation of an inactive ternary complex with DHFR and NADPH], inducing tetrahydrofolate (THF) deficiency. THF deficiency in turn leads to purine nucleotide and thymidine depletion, inhibiting nucleic acid synthesis. Thymidilate depletion is accentuated by inhibition of thymidilate synthetase by MTX-derived polyglutamates (associated with one or more glutamic acid molecules in the cells) [6].

Methotrexate absorption is complex. MTX uses an active transmembrane transport system, identical to that of tetrahydrofolate. This transport is saturable and, at high doses, passive diffusion may occur. Oral administration appears to be dose-dependent, although the product's bioavailability is quantitatively greater with higher doses. Subcutaneous or intramuscular administration is also possible, with peak blood levels reached in around 30 minutes.

In the circulation, 50-60% of the product is bound to albumin, and the drug is well distributed throughout the body. Only 1-3% of the plasma dose is found in the CSF, often necessitating intrarachid administration. Plasma kinetics are triphasic, with a peak in the first hour, followed by two much longer steps (around four hours, then more than 24 hours). The main metabolite of MTX is 7-hydroxy-methotrexate, produced by the action of a hepatic aldehyde oxidase. Long thought to be inactive, it now appears to form cytotoxic polyglutamates. Elimination is 90% renal, with only 10% biliary elimination when the protocol is intravenous. Elimination half-life is around 10 hours. Methotrexate (MTX) toxicity results from the combined effect of blood concentration and duration of exposure. It is manifested by anorexia, weight loss, hemorrhagic diarrhea, leukopenia and thrombocytopenia, liver disorders; in severe intoxications, comas may occur. Combinations with other drugs can have serious consequences: for example, those with non-steroidal anti-inflammatory drugs (NSAIDs) are absolute contraindications, as renal elimination is likely to be reduced. For the same reason, association with aminoglycosides is also inadvisable. Combination with trimethoprim-sulfamethoxazole would increase the risk of bone marrow toxicity. Pregnancy is of course contraindicated, due to the risk of fetal malformation [4,5]

## 2. Materials and Methods

### 2.1. Principle of the methotrexate assay

This is a one-step immunoassay for the quantitative determination of methotrexate in human serum and plasma using microparticle chemiluminescence immunoassay (CMIA) technology.

The sample, paramagnetic microparticles coated with anti-methotrexate antibodies, assay-specific diluent and acdidinium-labeled methotrexate conjugate are brought together to form a reaction mixture, then incubated. The methotrexate present in the sample binds to the anti-methotrexate antibody-coated microparticles and to the acridinium-labeled methotrexate conjugate. After a wash cycle, preactivation and activation solutions are added.

The resulting chemiluminescent reaction is measured in relative units of methotrexate light present in the sample and URLs detected by the optical system.

### 2.2. Verification procedure

This descriptive study was carried out over a 30-day period in the biochemistry laboratory of the CHU Mohammed VI Oujda. The working approach of our study was adapted based on the protocol of the COFRAC GTA 04 certification technical guide. Methotrexate was tested on the Alinity ci automated immunoassay system as part of method verification, to assess analytical performance in terms of repeatability. And reproducibility using samples from patients treated at CHU Mohammed VI Oujda, in addition to internal controls.

Data were statistically processed using BYG Informatics' EVM middleware module, a gateway application between the Alinity and iLAB results validation software.

Coefficient of variation (CV) values from our study were compared with those established by learned societies (FSCB and RICOS). Random subjects were selected from the regular workflow. No exclusion criteria were applied. Two phases of our research were carried out. The first stage concerned reproducibility through daily monitoring of the three levels: low, medium and high over a 30-day period. Methotrexate values were obtained from a group of serum samples in the next stage. Three sample groups were created: low, medium and high levels. 30 analyses of each sample were performed to assess repeatability.

## 3. Results

### 3.1. Repeatability results

Repeatability testing involves analyzing the same sample under optimal conditions to assess system performance and functionality.

CV values are again used to measure variability. For the low, medium and high levels, CV values are provided (CV1 = 1.99%, CV2 = 1.68%, CV3 = 2.71%). These results are illustrated on the Levey-Jennings graphs (Fig. 1, fig. 2, fig.3). Similar to the intermediate fidelity results, the FSBC limits and those established by the supplier with expansion factors are mentioned and the CV values are compared with these limits (Table 1).

In both cases, the conclusion is based on the comparison of CV values with specific tolerance limits. The CV values provided represent the variability observed in the measurements. The fact that the CV values calculated are below the specific limits suggests that the system's reproducibility and repeatability are within an acceptable range.

### 3.2. Reproducibility results

In the reproducibility test, the same sample is analyzed under different conditions to assess the impact of factors such as operators, time, reagent batches and calibrations on results. The Coefficient of Variation (CV) is used to measure the variability of results.

For low, medium and high levels, CV values are provided (CV1 = 3.34%, CV2 = 4.74%, CV3 = 4.57%). These results are illustrated on Levey-Jennings graphs (Fig. 4, fig. 5, fig.6). The concluding argument for each level is that the Reproducibility CV is correct and below the tolerated limit. In addition, the FSBC limits (a quality control system) and the limits set by the supplier with expansion factors are mentioned, and the CV values are compared with these limits (Table 2).

**Table 1** Repeatability results for methotrexate on Alinity automaton

| SAMPLE | N | MEANS (µg/ml) | SD | CV % | CV SUPPLIER% | CV RICOS |
|---|---|---|---|---|---|---|
| Level 1 | 30 | 0.07 | 0.001 | 1.99 | 11.25 | 2.1 |
| Level 2 | 30 | 0.42 | 0.007 | 1.68 | 7.50 | 2.7 |
| Level 3 | 30 | 0.94 | 0.025 | 2.71 | 7.50 | 2.6 |

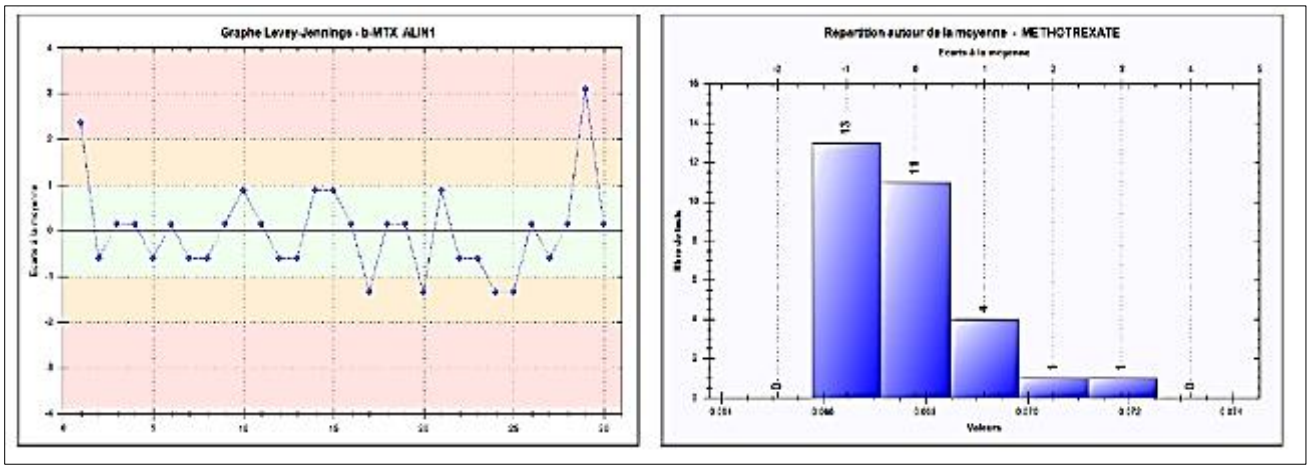


**Figure 1** Low level of repeatability, Levey Jenning graph and the distribution around the mean

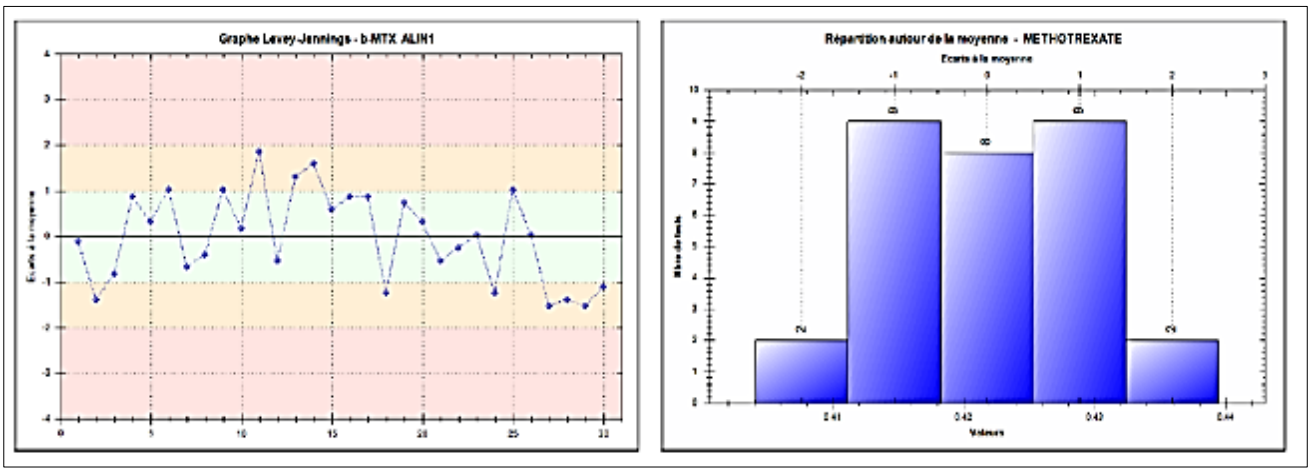


**Figure 2** Medium level of repeatability, Levey Jenning graph and the distribution around the mean

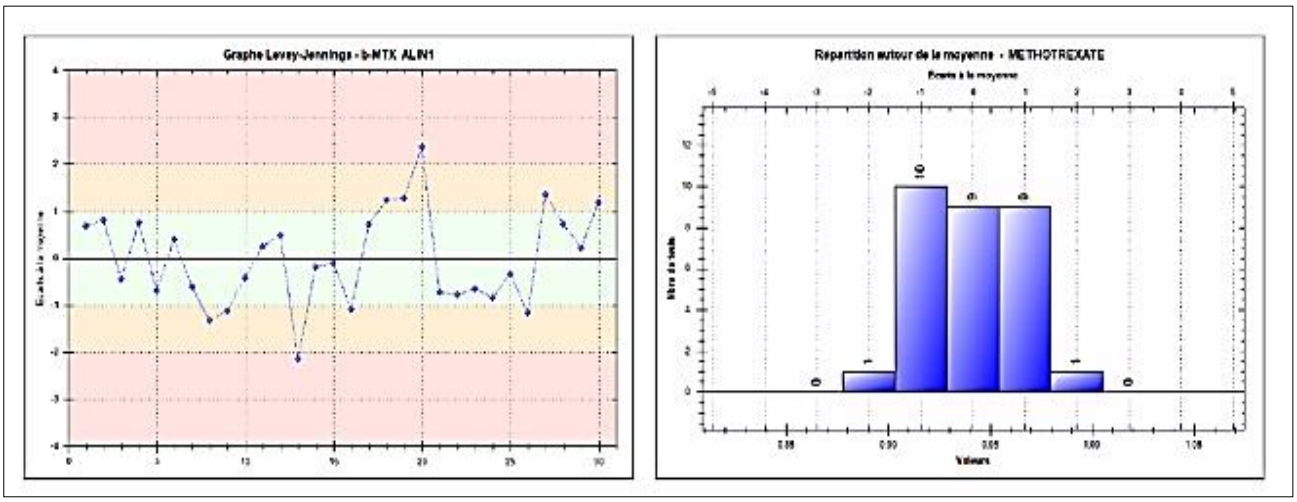


**Figure 3** High level of repeatability, Levey Jenning graph and the distribution around the mean

**Table 2** Reproducibility results for Sodium on Architect ci8200 automaton

| SAMPLE | N | MEAN (µg/dl) | SD | CV% | CV SUPPLIER% | CV RICOS% |
|---|---|---|---|---|---|---|
| Level 1 | 30 | 0,07 | 0,002 | 3,34 | 15 | 2 |
| Level 2 | 30 | 0,43 | 0,020 | 4,74 | 10 | 2,9 |
| Level 3 | 30 | 0,96 | 0,044 | 4,57 | 10 | 1,9 |

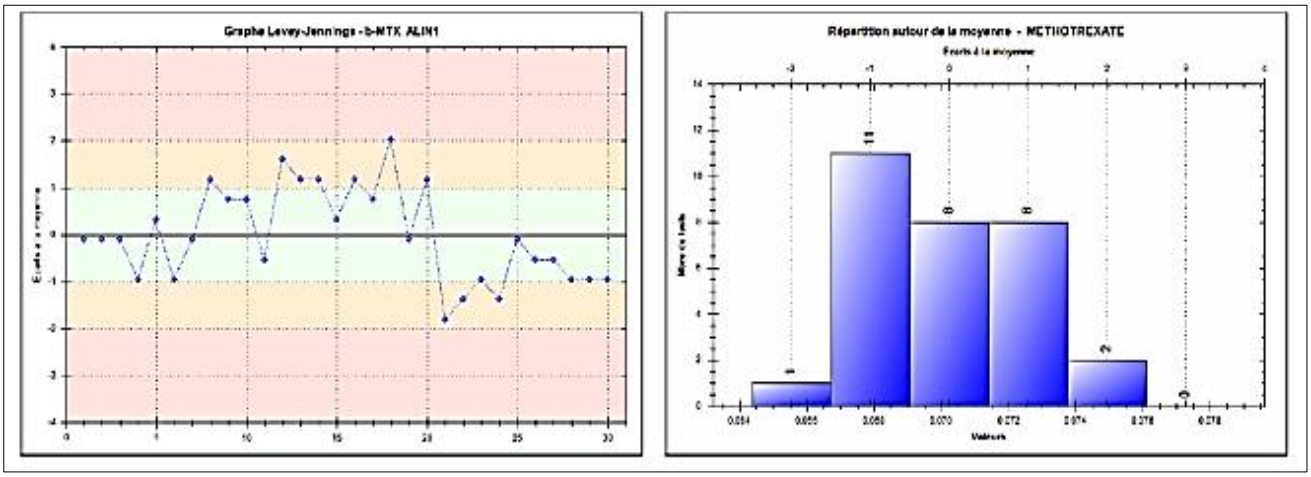


**Figure 4** Low level of reproducibility, Levey Jenning graph and the distribution around the mean

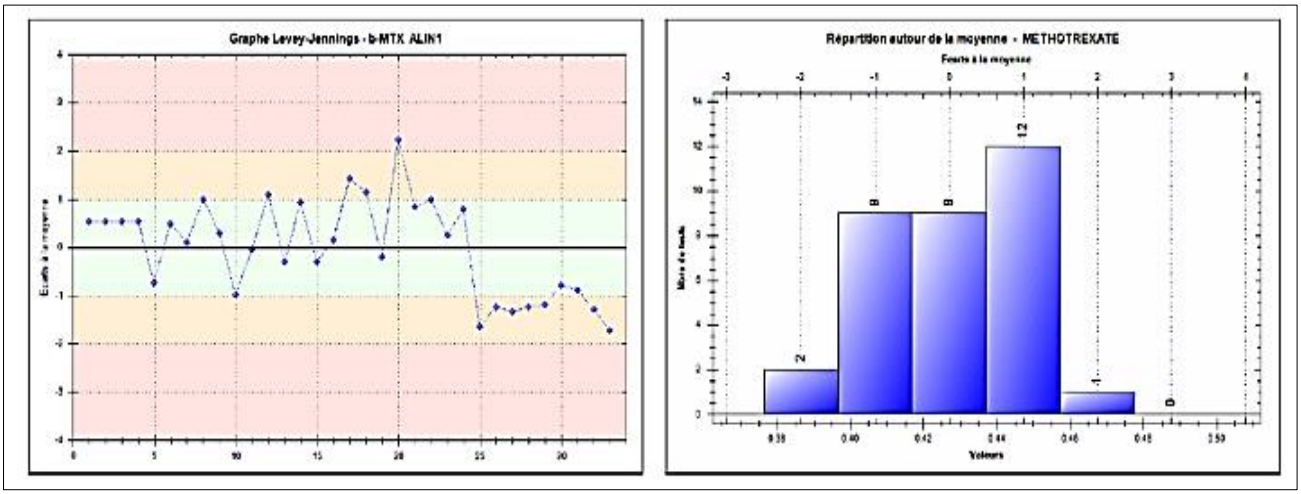


**Figure 5** Medium level of reproducibility, Levey Jenning graph and the distribution around the mean

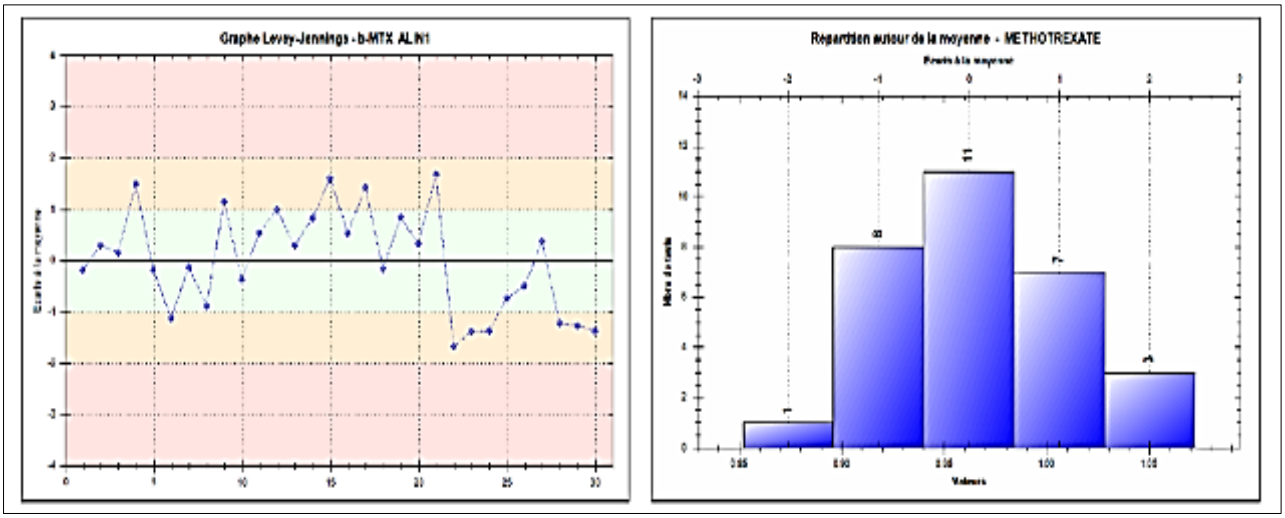


**Figure 6** High level of reproducibility, Levey Jenning graph and the distribution around the mean

## 4. Discussion

Methotrexate is a cytostatic antineoplastic of the antifolate group. It is marketed as 2.5 mg or 10 mg tablets, 5, 25, 50 or 100 mg solution for injection, or 10 mg/ml solution for injection in pre-filled syringes.

Methotrexate is widely used in the treatment of placental choriocarcinomas, mammary and ovarian adenocarcinomas, small-cell bronchial carcinomas, carcinomas of the upper aerodigestive tract, bladder carcinomas and, at high doses, in the treatment of childhood acute lymphoblastic leukemia, non-Hodgkin's malignant lymphomas and osteosarcomas. Methotrexate toxicity is essentially hematopoietic (thrombocytopenia, leukoneutropenia, more rarely anemia, agranulocytosis or pancytopenia), renal (increased creatinine levels) and hepatic (increased transaminases). In fact, before each methotrexate administration, it is essential to check the blood count and look for possible renal or hepatic insufficiency. Efficient management of methotrexate-based therapy requires rapid, selective and accurate measurement of methotrexate levels, both for diagnostic and monitoring purposes, and to avoid potential toxicity with far-reaching consequences. Methotrexate assessment is extremely important in medical practice, so an accurate result from the medical biology laboratory is essential, helping to preserve the laboratory's reputation and credibility. Control of the assay method used by the biologist in the laboratory is an ongoing concern, and its verification/validation is a regulatory (Moroccan guide to good performance in medical laboratory analyses) and normative (ISO 15189:2012) requirement [2]. By establishing predetermined analytical objectives, this control helps to generate accurate and reliable results. Reproducibility testing is used to critically evaluate the consistency of test results when different variables are introduced [1]. These include variations in operators, time, reagent batches and calibrations, all of which can have an impact on the reliability of results. To quantify this variability, the coefficient of variation (CV) is used. The CV provides a percentage measure of the deviation of results from the mean, indicating the level of dispersion of the data. For each of the low, medium and high levels, the CV values are 3.34%, 4.74% and 4.57% respectively. These values are relatively low, implying that the test produces consistent results under different conditions. The reproducibility results suggest that the immunological method (CMIA) performs well and is stable under a variety of conditions. The low CV values indicate that even when various factors are modified, such as the operator or reagent batch, the test consistently produces results close to the mean value. This reliability is crucial in medical testing, where consistency ensures that test results can be relied upon for clinical decisions. The fact that CV values align with established quality control limits indicates that the test meets industry standards for reproducibility, reinforcing its suitability for accurate diagnostic use. The repeatability test focuses on the accuracy of the test under controlled, optimal conditions. This is important because it assesses the method's ability to produce similar results when the same sample is analyzed several times [7]. The CV values for repeatability are remarkably low: CV1 = 1.99%, CV2 = 1.68% and CV3 = 2.71%. These values indicate extremely low variability, reaffirming the high precision of the test. The repeatability results suggest that the method (CMIA) provides consistent and highly accurate measurements when analyzing the same sample repeatedly. The exceptionally low CV values underline that the test results 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 CV values with quality control standards underlines the test's reliability and ability to generate reproducible results. Reproducibility and repeatability results collectively reinforce the robustness and reliability of the methotrexate immunoassay (CMIA) [8]. The test demonstrates low variability and high accuracy under

variable conditions and repeated analysis of the same sample. These qualities are paramount in clinical diagnostics, where accurate and reliable results are crucial to patient care. Comparison with quality control standards provides objective validation of test performance, reassuring researchers and healthcare professionals that the method produces consistent, reliable results. Meticulous evaluation of variability ensures that the test meets industry standards and can be used with confidence in clinical decision-making processes [9].

The laboratory of the CHU Mohammed VI d'Oujda is committed to a quality approach in order to satisfy the needs of its customers: patients, prescribers, samplers, both inside and outside the establishment. As part of its commitment to quality, the central laboratory has set up a method verification procedure and an accreditation process. The importance of these studies lies in their contribution to the establishment of a robust accreditation process for the laboratory's analyses, thus guaranteeing the reliability and accuracy of the results obtained.

## 5. Conclusion

In summary, meticulous evaluation of the analytical performance of the Abbott Alinity ci methotrexate assay using the CMIA immunological method reveals its robustness, reliability and accuracy. The alignment of CV values with quality control standards reinforces confidence in the test's consistency. Such reliable performance has substantial implications for healthcare, where the accuracy of diagnostic results has a direct impact on patient outcomes. This study testifies to the meticulous quality assurance processes implemented in the laboratory, and reaffirms the importance of verifying the analytical performance of clinical tests. Ultimately, this research contributes to the knowledge base underpinning the reliability of methotrexate level measurements, supporting clinical practice and patient safety.

## Compliance with ethical standards

### *Disclosure of conflict of interest*

The authors declare no conflict of interest.

### *Funding Sources*

This research did not receive any specific funding from public, commercial, or non-profit funding agencies.

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