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Verification of analytical performance of transferrin assay 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 essential to ensure the accuracy of results. This study evaluates the performance of the transferrin assay method in the central laboratory of CHU Mohammed VI in Oujda, in order to confirm its reliability for the diagnosis and monitoring of iron metabolism disorders.

We conducted a performance study of the Alinity ci® system by assessing repeatability and reproducibility in accordance with the COFRAC GTA 04 technical accreditation guidelines, in line with the quality requirements of the ISO 15189 standard.

The results of our study indicate satisfactory repeatability for the three levels (low, medium, and high), with coefficients of variation of 1.64%, 1.13%, and 1.10%, respectively. Additionally, reproducibility was also satisfactory, with coefficients of variation for these same levels of 2.88%, 2.65%, and 3.30%, respectively.

The reliability of transferrin assay results in our laboratory is confirmed by the satisfactory results obtained in our study, which comply with the recommendations of RICOS and FSCB.

Keywords: Transferrin; Analytical Performance; Repeatability; Reproducibility; Alinity CI Analyzer

1. Introduction

Quality is a continuous process focused on ensuring that tests are performed accurately and consistently. A quality assurance system includes all internal and external laboratory activities, along with adherence to proper practices and enhanced management skills. Its goal is to ensure that each assay conducted by the laboratory is both accurate and reliable. To implement quality concepts in medical laboratories, it is essential to establish a targeted quality management process that maintains the reliability of results [1].

The verification of analytical methods is essential for evaluating and ensuring the performance, reliability, and precision of the methods used in the laboratory. This process involves following a standardized operating protocol, as well as assessing against criteria defined by learned societies such as RICOS and FSCB, as well as by the manufacturer. In addition to meeting regulatory requirements outlined in the Moroccan Guide for the Good Performance of Medical Laboratory Analysis (GBEA) and the ISO 15189:2022 standard, this verification allows laboratories to gain an in-depth understanding of their analytical methods, their performance, and their limitations, thereby ensuring the accuracy of results for the benefit of patients and prescribers. Therefore, it is crucial to ensure that these performances are adequate [2,3,4].

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Iron is crucial for numerous metabolic pathways and physiological functions [5] Maintaining iron homeostasis is important, as both deficiencies and excesses can be detrimental to the body. Transferrin, which has a strong affinity for ferric iron, binds almost all plasma iron, resulting in minimal free iron in the body. This blood plasma glycoprotein plays a key role in iron metabolism and is responsible for transporting ferric ions. Transferrin serves as the primary reservoir of ferric iron, delivering iron to various tissues, including the liver, spleen, and bone marrow. It is also a vital biochemical marker for assessing the body's iron status [6].

Our study focuses on the in-depth analysis of the analytical performance of the transferrin test on the Abbott Alinity ci automated system. We have established a method verification protocol for this test, examining several key parameters in detail, such as precision, accuracy, and reliability of measurements. This analysis is crucial not only for strengthening laboratory practices and improving diagnostic approaches but also for serving as an essential foundation for an accreditation procedure, demonstrating our commitment to a rigorous quality process within our laboratory.

1.1. Transferrin and clinical Significance

Iron deficiency is recognized as the most common nutritional deficiency worldwide. The level of transferrin in the blood serves as an indicator of the body's iron status. Elevated transferrin levels typically signify low iron availability, indicating that less iron is bound to transferrin, which results in increased circulation of unbound transferrin and may suggest the presence of iron deficiency anemia [7] To maintain homeostasis, the liver enhances transferrin production to facilitate the binding and transport of iron to cells. In cases of iron deficiency anemia, there is an upregulation of transferrin receptors.

Regarding the transferrin-iron complex, a lower percentage of iron-bound transferrin reflects insufficient iron levels in the body, which can negatively impact hemoglobin production and erythropoiesis. The measurement of transferrin is significant for detecting iron deficiency and monitoring erythropoiesis. In the context of anemia of chronic disease, transferrin levels tend to be lower.

Factors contributing to decreased transferrin include:

- Liver damage, which reduces transferrin production
- Kidney injury or dysfunction, leading to transferrin loss in urine
- Infections
- Malignancies
- Atransferrinemia: A genetic mutation causing the absence of transferrin, which can result in hemosiderosis in the heart and liver, potentially leading to heart and liver failure. This condition is typically managed through plasma infusion.

Additionally, low transferrin levels in plasma can indicate iron overload, suggesting that transferrin binding sites are heavily saturated with iron. This condition may be indicative of hemochromatosis, leading to iron deposition in tissues. Other associations with transferrin and its receptors include:

- The reduction of tumor cells when the receptor is employed to attract antibodies.
- Increased transferrin saturation is linked to a higher risk of cardiovascular mortality, particularly in patients with transferrin saturation exceeding 55% and elevated LDL levels[8].

1.2. Principle of the assay method

The Transferrin assay is an automated clinical chemistry test that utilizes an immunoturbidimetric procedure. This method quantifies the increasing turbidity of the sample, which results from the formation of insoluble immune complexes when transferrin antibodies are introduced. Initially, the sample containing transferrin is incubated with a buffer (R1), and a sample blank is determined before adding the transferrin antibody (R2). The concentration of transferrin is then measured based on the turbidity, with the antibody present in excess to ensure accurate results.

2. Material and methods

This study is a prospective investigation conducted within the biochemistry laboratory of Mohammed VI University Hospital. Our primary objective was to assess the analytical performance of transferrin determination using the "Chemistry" module on the Abbott Alinity ci® Analyzer. The study was carried out over a specified period and adhered to the recommendations outlined in the accreditation technical guide of the French accreditation committee (COFRAC),

protocol GTA 04. The findings from this study will provide essential insights into the accuracy and reliability of transferrin determination in a hospital setting.

The study was structured into two distinct phases. Initially, we evaluated the intermediate fidelity, also known as intra-laboratory reproducibility, by conducting daily internal quality controls with samples at varying concentrations—low, medium, and high—over a 30-day period. In the second phase, we assembled a comprehensive collection of serum samples, ensuring an equitable distribution of transferrin values across the entire measurement spectrum. These collected samples were categorized into two groups representing low and high transferrin levels. To assess repeatability, each serum sample underwent 30 individual assay runs.

The transferrin evaluation utilized a dedicated reagent kit on the chemistry module. Subsequently, data processing was performed via the BYG middleware, which connects the Alinity platform to the iLab result validation software. The coefficient of variation (CV) values obtained from this study were then compared to the reference standards established by respected professional organizations and the supplier, specifically the Federation of Clinical Chemistry and Laboratory Medicine (FSCB) and the Reference Institute for Bioanalytics (RICOS).

3. Results

3.1. Reproducibility results

The intra-laboratory reproducibility, involves examining the same sample under varying conditions by altering at least one variable, such as the operator, time, reagent batches, or calibration settings. This method helps establish acceptance criteria based on existing knowledge, particularly considering biological variations in decision support systems. By testing the sample under different conditions and carefully analyzing the resulting outcomes, researchers can evaluate how various factors affect the test's accuracy and reliability. This approach enhances understanding of the test's robustness and performance, ultimately aiding in the development and optimization of diagnostic methods and improving the overall quality of laboratory analyses in clinical diagnostics. (9)

Table 1 Reproducibility results of blood assays by level compared to the manufacturer's specifications**.**

Level of Internal quality control (IQC)	Number of values	Mean (g/l)	Standard Deviation (g/l)	Coefficient of Variation CV (%)	CV SFBC 1999 (%)	CV RICOS 2014 (%)
Low level	30	1.77	0.051	2.88	8.00	1.50
Medium level	30	2.54	0.067	2.65	6.00	1.50
High level	30	3.57	0.118	3.30	5.00	1.50

The results of our study indicate satisfactory reproducibility, with coefficients of variation for these levels of 2.88%, 2.65%, and 3.30%, respectively.

To enhance clarity regarding these results, they have been visually illustrated using Levey-Jennings plots (Fig. 1, Fig. 2, and Fig. 3).

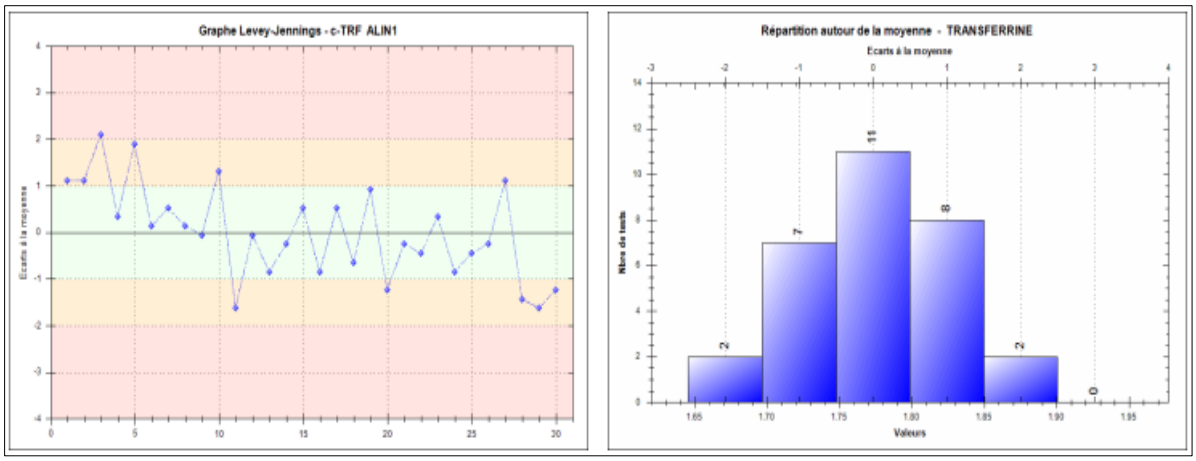

Figure 1 Low level of reproducibility: Levey Jennings graph and the distribution around the mean

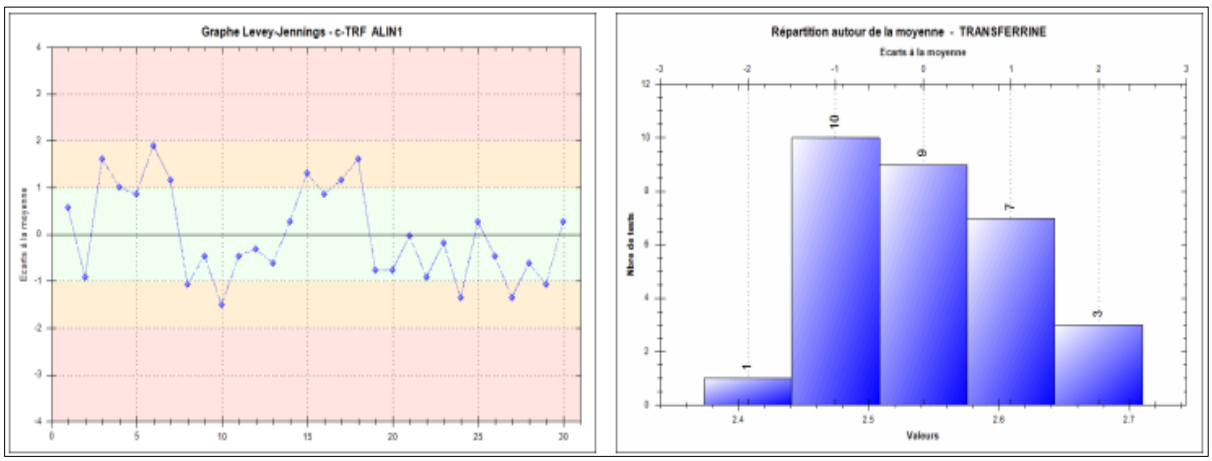

Figure 2 Medium level of reproducibility: Levey Jennings graph and the distribution around the mean

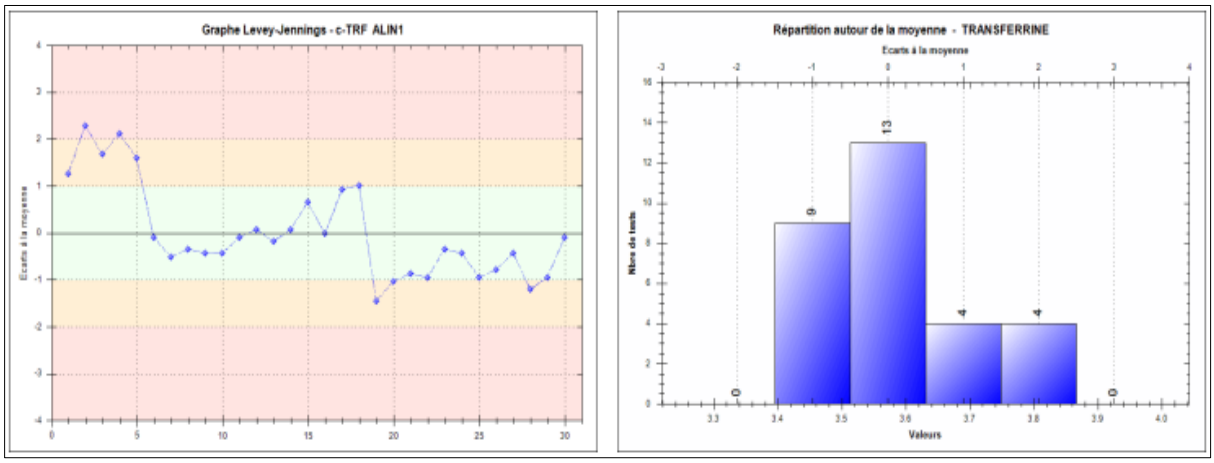

Figure 3 High level of reproducibility: Levey Jennings graph and the distribution around the mean

3.2. Repeatability results

Repeatability is evaluated by having the same operator conduct repeated assays on identical samples under consistent conditions, accounting for all measurement factors, including reagent, calibration, instrument, and operator, while minimizing the time between tests. This repeatability assessment helps establish initial performance and verifies the

proper functioning of the system (instrument/reagent) for the specific analyte. (10) Additionally, variability is quantified using coefficient of variation (CV) values.

Table 2 Repeatability results for transferrin on the Alinity i® automated system by level, compared to the manufacturer's specifications.

Level of Internal quality control (IQC)	Number of values	Mean (g/l)	Standard Deviation (g/l)	Coefficient of Variation CV (%)	CV SFBC 1999 (%)	CV RICOS 2014 (%)
Low level	30	1.74	0.029	1.64	6.00	1.13
Medium level	30	2.44	0.028	1.13	4.50	1.13
High level	30	3.44	0.038	1.10	3.75	1.13

The results of our study indicate satisfactory repeatability for the three levels (low, medium, and high), with coefficients of variation of 1.64%, 1.13%, and 1.10%, respectively.

These findings are displayed using Levey-Jennings plots, providing a clearer illustration of the results (Fig. 4, Fig. 5, and Fig. 6).

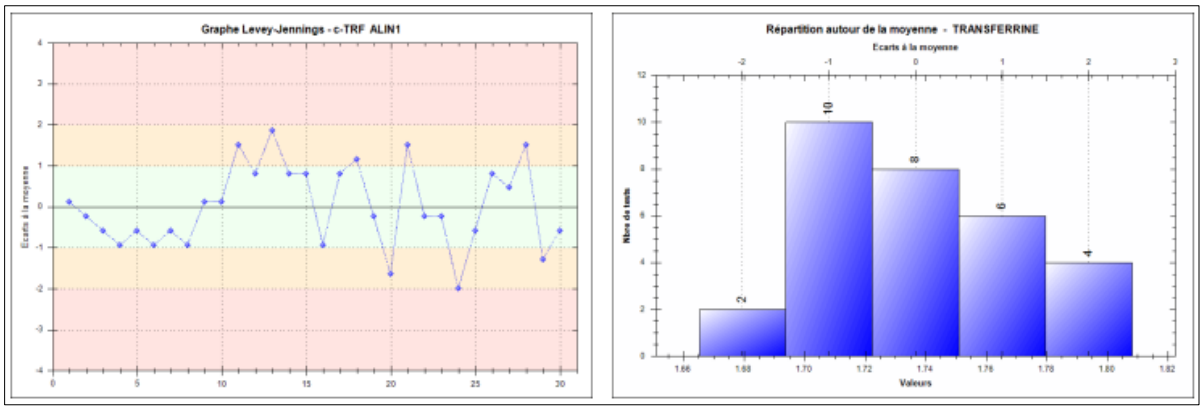

Figure 4 Low level of repeatability: Levey Jennings graph and the distribution around the mean

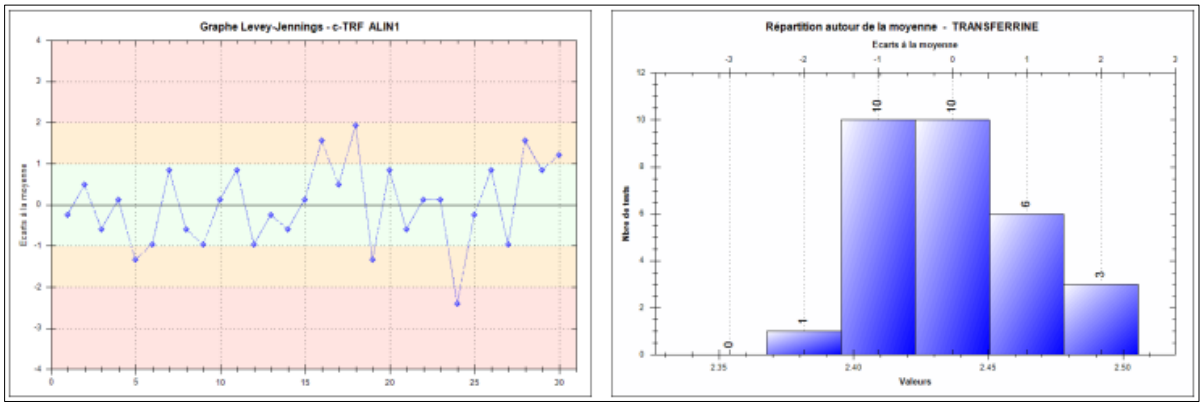

Figure 5 Medium level of repeatability: Levey Jennings graph and the distribution around the mean

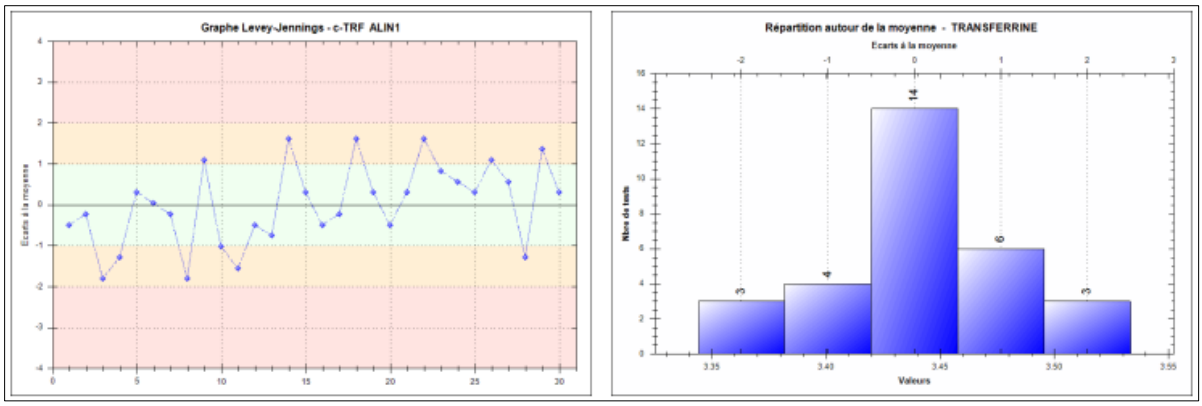

Figure 6 High level of repeatability Levey Jennings graph and the distribution around the mean

4. Discussion

Immunological methods for measuring serum transferrin (TF) concentration are now available. Earlier immunodiffusion techniques (11) have been largely replaced by automated methods such as immunoturbidimetry (12) or immunonephelometry (13). Determining serum TF has some technical advantages, including the need for a smaller sample volume. Recently, CRM 470 standardization was adopted for fourteen plasma proteins (14), with the reference range for TF reported as 2.0–3.6 g/l. Reference intervals were also established in a Japanese population using the same standardization (15), with similar results (1.90–3.20 g/l), suggesting that racial differences are not highly pronounced. The introduction of international reference materials for serum proteins has led to a significant reduction in between-laboratory variability for TF (16). Coefficients of variation are very low, well below the requirements considering the large biological variation of iron (17). International interim reference ranges for various plasma proteins, including TF (18), have been proposed and accepted by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and many national scientific societies.

Mastering the analytical method used by laboratory personnel is an ongoing priority, and its verification or validation is both a regulatory requirement (as outlined in the Moroccan Guide for the Good Performance of Medical Laboratory Analyses) and a standard requirement (according to ISO 15189:2012) (19). By setting predefined analytical goals, this mastery ensures the production of accurate and reliable results.

The reproducibility test is an essential tool for evaluating the consistency of assay results when subjected to varying conditions. These include changes in operators, time intervals, reagent batches, and calibration processes, all of which can affect the reliability of the outcomes. To measure this variability, the Coefficient of Variation (CV) is employed. The CV reflects the degree to which the results deviate from the average, indicating how much the data disperses. For the low, medium, and high levels, the CV values were 1.64%, 1.13%, and 1.10%, respectively, which are relatively low, suggesting that the assay produces consistent results across varying conditions.

The reproducibility results indicate that the transferrin assay is stable and reliable, even when various factors such as the operator or reagent batch are altered. This stability is critical in clinical testing, where it is essential that results are consistent and can be trusted for decision-making. Furthermore, the CV values fall within the expected quality control limits, affirming that the assay meets industry standards for reproducibility, making it suitable for accurate diagnostic applications.

The repeatability test, on the other hand, examines the precision of the assay when performed under controlled and optimal conditions. This test evaluates how consistently the method delivers the same results when the same sample is analyzed multiple times. The CV values for repeatability are low: 2.88%, 2.65%, and 3.30%. These values indicate that the variability is minimal, confirming the high precision of the assay.

The repeatability results show that the transferrin assay provides highly precise and consistent measurements when the same sample is analyzed repeatedly. The low CV values highlight the stability and predictability of the assay under controlled conditions. This precision is particularly important in clinical diagnostics, where even minor variations can significantly impact patient care. The alignment of the CV values with quality control standards further validates the reliability and suitability of the assay for generating repeatable and accurate results.

Together, the reproducibility and repeatability results emphasize the robustness and reliability of the transferrin assay. The assay demonstrates low variability and high precision across different conditions and repeated analyses of the same sample. These attributes are crucial in clinical diagnostics, where dependable and accurate results are vital for patient care. The comparison with quality control standards provides an objective validation of the assay's performance, assuring healthcare professionals and researchers that the method delivers consistent, trustworthy results. This thorough evaluation of variability ensures that the assay complies with industry standards and can be confidently used in clinical decision-making.

5. Conclusion

The analytical performance of the Alinity CI automated system was satisfactory for reliable transferrin determination. Verification of assay methods in medical laboratories is critical to ensuring the accuracy, precision, and reliability of test results. This process involves confirming that the test method used is appropriate for its intended purpose, delivers results consistent with the claimed performance characteristics, and meets the laboratory's quality control and assurance requirements.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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