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Verification of the analytical performance of free triiodothyronine on ALINITY ci ® experience from the biochemistry laboratory of Mohammed VI University Hospital in 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 comparing it with pre-established analytical objectives. In our study, we conducted an evaluation of the analytical performance of the free triiodothyronine (FT3) assay using the Abbott kit on the Alinity ci® analyzer in the biochemistry laboratory of Mohammed VI University Hospital in Oujda. The methodology adhered to the recommendations of the French accreditation committee (COFRAC) technical guide SH GTA 04, focusing on the assessment of repeatability and reproducibility. The results demonstrated excellent precision across low, medium, and high FT3 concentrations, with coefficients of variation (CV) within acceptable limits. These findings confirm the reliability of the Alinity ci® system for FT3 measurement, supporting its use in clinical diagnostics. It is important to note that the accuracy and reliability of examination results depend not only on laboratory personnel, equipment, and environmental conditions but also on the methods utilized and their validation or verification.

Keywords: Free Triiodothyronine (FT3); Analytical Performance; Repeatability; Reproducibility; Alinity CI Analyzer; Immuno-Chemiluminescence

1. Introduction

Free triiodothyronine (FT3) is a crucial biomarker in thyroid function assessment. It is the biologically active form of thyroid hormone, playing a key role in regulating metabolism, growth, and development. FT3 is primarily produced through the peripheral conversion of thyroxine (T4), its levels can be altered in various thyroid disorders, including hyperthyroidism and certain cases of hypothyroidism. Given its significance in both endocrinology and clinical diagnostics, precise and reliable measurement techniques are essential.

With advancements in laboratory methodologies, the use of chemiluminescent technology in FT3 assays has emerged as a promising approach, offering enhanced sensitivity and accuracy in detecting FT3 levels. Our study focuses on the critical process of method verification for the FT3 assay using immunochemiluminescence technology implemented on the Abbott Alinity ci analyzer. This verification involves a thorough assessment of analytical performance, adhering to standardized operational procedures and comparing results against established criteria set by recognized organizations (RICOS, FSCB). This comprehensive approach provides laboratories with essential insights into the assay's capabilities and limitations, ensuring that it meets the standards necessary for delivering accurate, reliable, and clinically relevant results.

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1.1. Free triiodothyronine (FT3) and dosing interests

3,5,3'-Triiodothyronine (T3) is a thyroid hormone with a molecular weight of 651 daltons [1] and a serum half-life of 1.5 days [2].

T3 circulates in the blood as a free hormone in equilibrium with its protein-bound form [3]. It is primarily bound to thyroxine-binding globulin (TBG), prealbumin, and albumin. However, the actual binding rates of T3 to these proteins remain controversial, with estimates ranging from 38% to 80% for TBG, 9% to 27% for prealbumin, and 11% to 35% for albumin [4].

Due to the high binding capacity of these proteins, only 0.2% to 0.4% of total T3 exists in solution as free, unbound T3 [5]. This free fraction represents the physiologically active thyroid hormone [3].

In Graves' disease, the increase in free T3 levels is more significant than that of free thyroxine (T4) [6,7]. In approximately 5% of hyperthyroid patients, only free T3 is elevated, a condition known as T3 toxicosis [8]. Conversely, free T4 levels rise more significantly than free T3 in toxic multinodular goiter and in cases of excessive T4 therapy [9].

Serum free T3 measurement is useful for differentiating between these forms of hyperthyroidism. Free T3 testing is also important for monitoring patients undergoing antithyroid therapy, which aims to reduce T3 production and the conversion of T4 to T3. Additionally, free T3 serum determination helps assess the severity of thyrotoxic conditions.

1.2. Principle of the assay method

This assay is a two-step immunoassay for the quantitative determination of free triiodothyronine (Free T3) in human serum and plasma, using chemiluminescent microparticle immunoassay (CMIA) technology.

The sample is incubated with paramagnetic microparticles coated with anti-T3 antibodies. Free T3 present in the sample binds to the antibody-coated microparticles. After a washing step, acridinium-labeled T3 conjugate is added to form a reaction mixture, followed by incubation. Another wash cycle is performed before the addition of pre-activation and activation solutions.

The resulting chemiluminescent reaction is measured in relative light units (RLU). There is an inverse relationship between the amount of free T3 in the sample and the RLU detected by the optical system.

2. Material and methods

This study is a prospective investigation conducted over a 32-day period within the biochemistry laboratory of Mohammed VI University Hospital. The methodology adopted follows the recommendations outlined in the French accreditation committee (COFRAC) technical guide GTA 04. The study was structured into two distinct phases.

The first phase focused on assessing the reproducibility of results by performing daily tests on control samples at three concentration levels—low, medium, and high—throughout the 30-day period. The primary objective was to evaluate the assay’s consistency and reliability.

In the second phase, a diverse collection of serum samples was gathered, ensuring a balanced distribution of free triiodothyronine (FT3) values across the full measurement range. These samples were categorized into three groups corresponding to low, medium, and high FT3 levels. To evaluate repeatability, each serum sample underwent 33 individual assay runs. The FT3 quantification was performed using a dedicated reagent kit on the immunology module of the Abbott Alinity ci analyzer. Data processing was subsequently carried out via the BYG middleware, acting as an interface between the Alinity platform and the iLab result validation software.

The coefficient of variation (CV) values obtained from this study were then compared against the reference standards set by recognized professional organizations, including the Federation of Clinical Chemistry and Laboratory Medicine (FSCB) and no CV reference values were estabilished by the Reference Institute for Bioanalytics (RICOS). The findings of this investigation are detailed in the sections that follow.

3. Results

3.1. Repeatability Results

The results obtained from this investigation exhibited commendable levels of repeatability for all levels low, medium, and high concentration ranges, as indicated by CV1 of 1.39%, CV2 of 2.20%, and CV3 of 2.32% respectively (Table 1).

These findings have been graphically presented using Levey-Jennings plots to further illustrate the obtained results (Figures 1,2 and 3).

Table 1 Repeatability results for FT3 on the Alinity i® automated system by level with comparison to SFBC data

Levels	N	Mean	Standard deviation (SD)	CV %	CV% (FSCB)
LOW	33	5.39 pmol/ml	0.102 pmol/ml	1.90 %	8.25 %
MEDIUM	33	7.67 pmol/ml	0.103 pmol/ml	1.35 %	6 %
HIGH	33	14.22 pmol/ml	0.396 pmol/ml	2.79 %	6 %

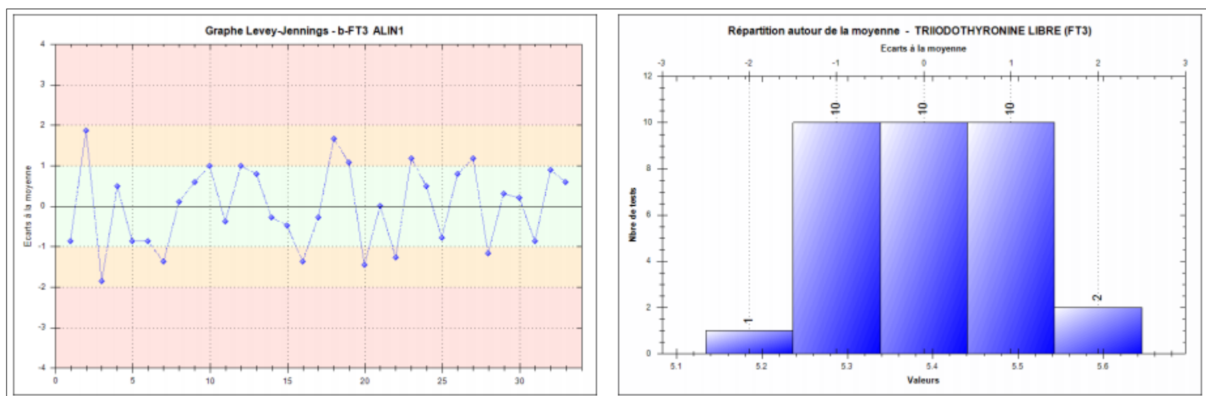

Figure 1 Low Level of Repeatability of FT3: Levey Jennings graph and the distribution around the mean

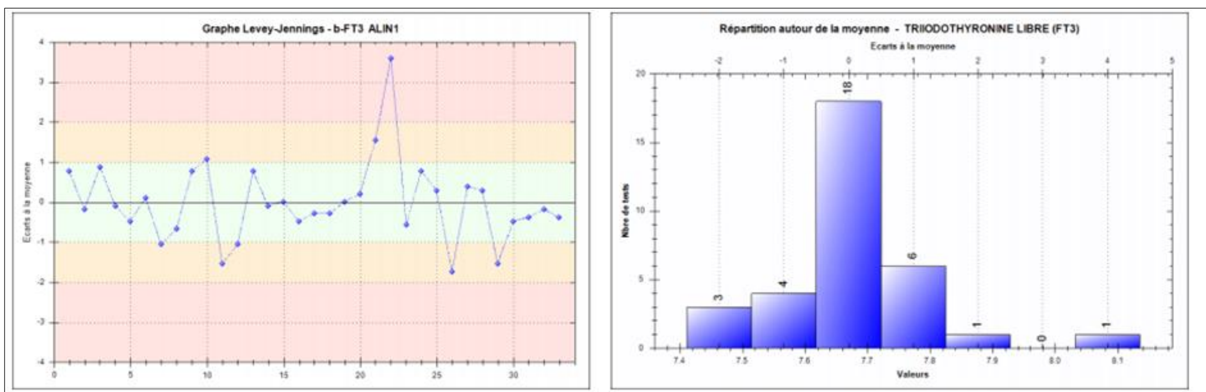

Figure 2 Medium Level of Repeatability of FT3 : Levey Jennings graph and the distribution around the mean

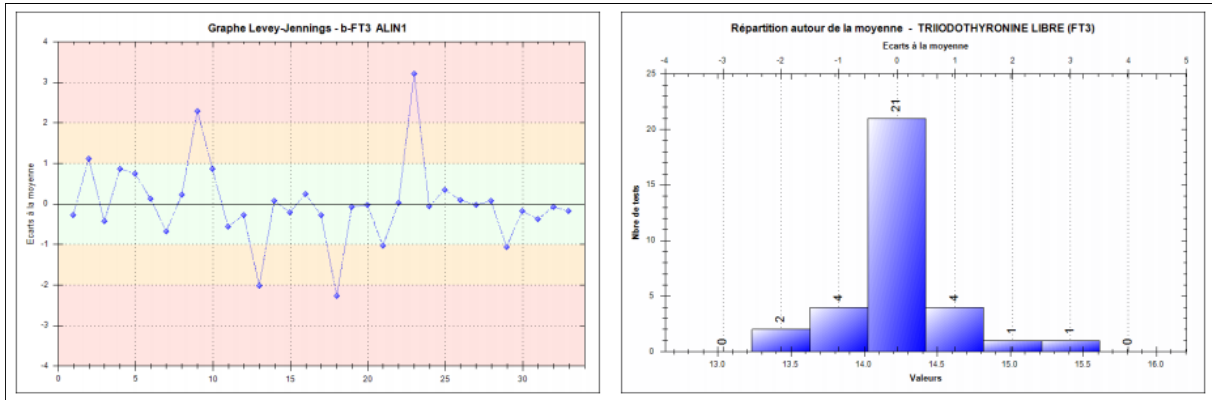

Figure 3 High Level of Repeatability of FT3 : Levey Jennings graph and the distribution around the mean

3.2. Reproducibility results

The reproducibility test assesses the same sample under different conditions to determine the influence of variables such as operators, time, reagent batches, and calibrations on the results. Its primary objective is to establish acceptance criteria, especially for decision support systems. Variability is measured using the Coefficient of Variation (CV). [10]

For the low, medium, and high levels, the CV values are provided (CV1 = 4.08 %, CV2 = 6.73%, CV3 = 5.55 %) These results are illustrated on the Levey-Jennings graphs (Fig. 5,6 and 7fig).

Table 2 Reproducibility results for FT3 on the Alinity i® automated system by level with comparison to SFBC data

Levels	N	Mean	Standard deviation (SD)	CV %	CV% (SFBC)
LOW	32	3.98 pmol /ml	0.162 pmol/ml	4.08 %	11 %
MEDIUM	32	10.61 pmol/ml	0.714 pmol/ml	6.73 %	8 %
HIGH	32	19.79 pmol/ml	1.098 pmol/ml	5.55 %	8%

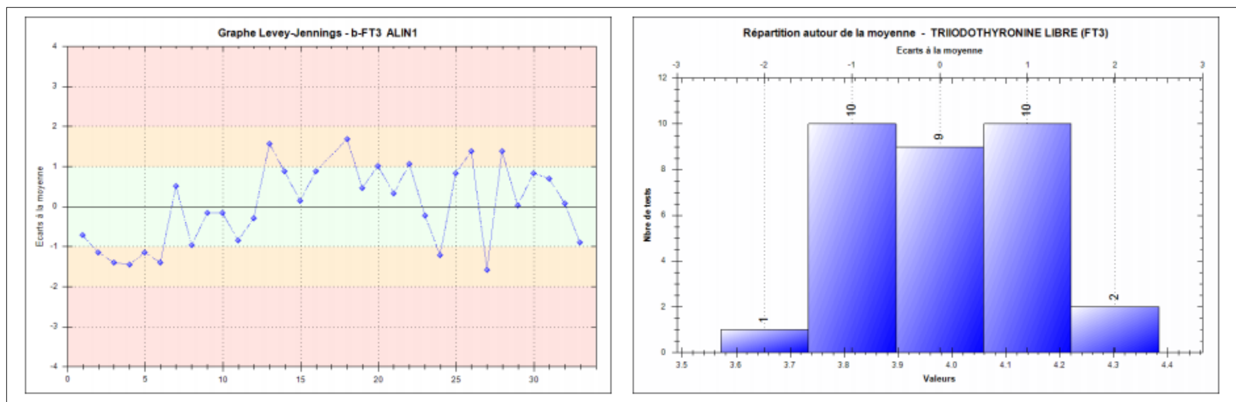

Figure 4 Low level of reproducibility of FT3 : Levey Jennings graph and distribution around the mean

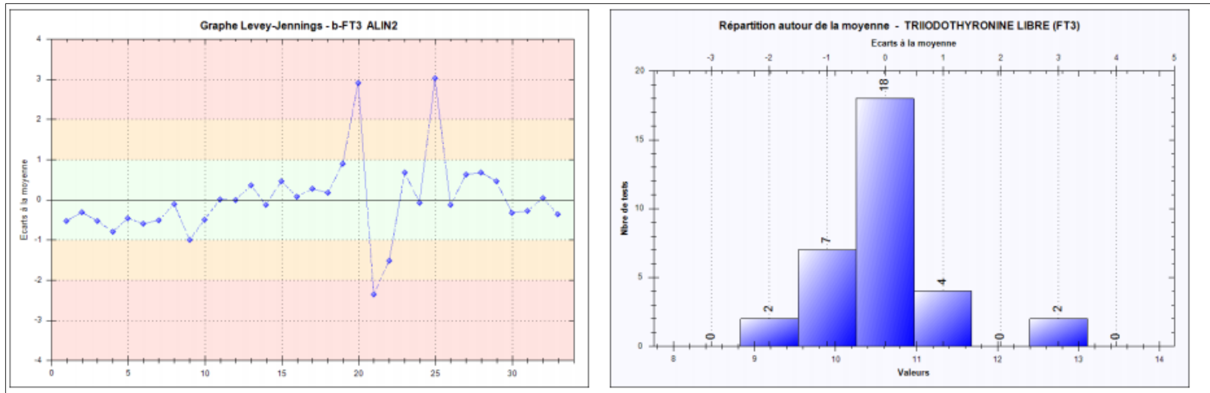

Figure 5 Medium level of reproducibility of FT3 : Levey Jennings graph and distribution around the mean

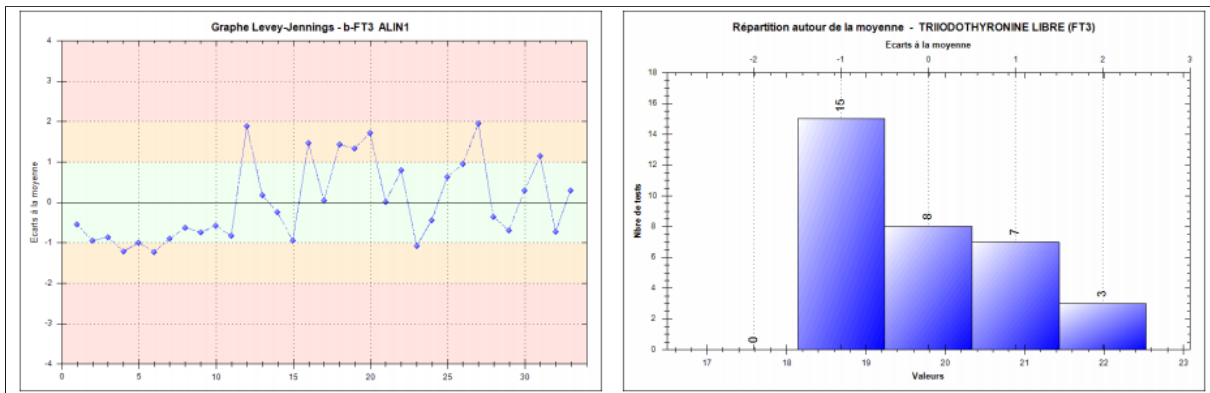

Figure 6 High level of reproducibility of FT3 : Levey Jennings graph and distribution around the mean

4. Discussion

Accurate diagnosis and treatment monitoring of thyroid disorders rely on measuring thyroid hormones, including triiodothyronine (T3), thyroxine (T4), and thyroid-stimulating hormone (TSH). While the thyroid gland produces all circulating T4 and approximately 20% of T3, the majority of T3 is generated through the enzymatic conversion of T4 by deiodinase in peripheral tissues. Most T3 and T4 in the bloodstream are bound to plasma proteins such as thyroxine-binding globulin (TBG), transthyretin (TTR), and albumin, with only a small fraction existing in the free, unbound form—0.3% for T3 and 0.02% for T4. This low concentration of free hormones makes their measurement technically challenging. [11]

For over three decades, assays to measure free T3 (FT3) and free T4 (FT4) have been developed, primarily using immunoassay (IA) methods or physical separation techniques. Immunoassays are conducted in the presence of protein-bound hormones, while physical separation methods, such as equilibrium dialysis, ultrafiltration, or gel filtration, isolate free hormones from their protein-bound counterparts [12,13]. Due to the complexity and cost of physical separation methods, most clinical laboratories opt for direct analogue immunoassays, which are performed on automated platforms. However, these immunoassays are influenced by binding proteins and are prone to various interferences, including drug interactions, antibody cross-reactivity, sensitivity to abnormal binding proteins, free fatty acid (FFA) effects, and the presence of endogenous or exogenous binding inhibitors. Consequently, concerns have been raised regarding the accuracy and reliability of direct analogue immunoassays. [12,14]

Immunoassays typically use radioactive, fluorescent, or chemiluminescent labels for hormone quantification. In recent years, advanced techniques such as gas chromatography-mass spectrometry (GC-MS), isotope dilution liquid chromatography-mass spectrometry (LC-MS), and tandem mass spectrometry (LC-MS/MS) have been employed to measure total T3 (TT3) and total T4 (TT4) in serum or plasma. These mass spectrometry methods offer superior specificity and reduced interference compared to immunoassays. Quantification relies on factors such as retention time, the presence of parent and fragmented ions, and the ratio of the analyte signal to that of a stable isotope. [12, 15,16]

The central laboratory of Mohammed VI University Hospital in Oujda is dedicated to maintaining the highest standards of analytical performance, adhering to NF ISO 15189 and COFRAC guidelines for method validation and verification. These processes are essential to ensure the reliability, accuracy, and reproducibility of results, which are critical for clinical diagnostics and patient care. As part of its quality assurance strategy, the laboratory undertakes method verification and validation to assess analytical performance parameters, including precision, reproducibility, and repeatability. These evaluations are conducted in line with international accreditation standards and aim to confirm that the methods used meet stringent quality control requirements. [17,18]

Intermediate fidelity testing evaluates the robustness of an assay under varying conditions, such as different operators, reagent batches, and calibration protocols. Results are assessed using statistical measures like coefficients of variation (CV), which must align with established quality limits to demonstrate reliability across diverse scenarios. Repeatability assessments focus on evaluating the consistency of results when the same sample is analyzed under identical conditions. This process ensures minimal variability and confirms the precision of the method, which is crucial for maintaining trust in clinical outcomes. [19]

By rigorously verifying and validating analytical methods according to strict standards, the laboratory ensures the delivery of accurate and consistent results. These efforts strengthen the laboratory's role as a reference center, enhance its accreditation readiness, and contribute to advancements in medical diagnostics and patient care. Such rigorous quality assurance processes reflect the laboratory's commitment to excellence and its pivotal role in supporting healthcare providers and improving patient outcomes. [20]

5. Conclusion

Verifying analytical methods in medical laboratories is a critical process to guarantee the accuracy, precision, and reliability of test results. This verification confirms that the chosen method is fit for its intended purpose, delivers consistent and reproducible results, aligns with claimed performance characteristics, and meets stringent quality control and quality assurance standards. In our study, the verification process for the FT3 assay adhered to COFRAC guidelines (GTA 04) and international standards, ensuring the method's robustness and suitability for clinical diagnostics. The results demonstrated excellent repeatability and reproducibility across low, medium, and high FT3 concentrations, with coefficients of variation (CV) well within acceptable limits. This study underscores the reliability of the Alinity ci® system for FT3 measurement, reinforcing its role in supporting accurate thyroid function assessment and enhancing patient care outcomes.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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