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(RESEARCH ARTICLE)

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Verification of the analytical performance of thyroglobulin autoantibodies on ALINITY ci ® experience from the biochemistry laboratory of Mohammed VI University Hospital in Oujda

Imane Douichi [1, 2, *], Asmae Kidoun [1, 2], Mohamed Khalil Bourchid [1, 2], Sara Moulay Rchid [1, 2], Meryem El Halas [1, 2], Dounia El Moujtahide [1, 2], El Houcine Sebbar [1, 2] and Mohammed Choukri [1, 2]

[1] *Mohammed First University, Faculty of Medicine and Pharmacy of Oujda, Morocco.*
[2] *Biochemistry laboratory of Mohammed VI University Hospital, Oujda, Morocco.*

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Abstract

The objective of this study was to assess the analytical performance of thyroglobulin autoantibody determination using a two-step immunoassay based on microparticle chemiluminescence immunoassay (CMIA) technology. This evaluation was conducted in compliance with the Scope A criteria outlined in the guidelines for the verification and validation of medical biology methods.

We investigated the assay's repeatability and intermediate precision. The results were highly satisfactory across three concentration levels (low, medium, and high). For intermediate precision, the coefficients of variation (CV) were:

CV1 = 2.56 %, CV2 = 3.60 % and CV3= 2.70 %, respectively. For repeatability, the coefficients of variation were CV1 = 3.13 %, CV2 = 2.90 and CV3= 2.40 %, respectively.

These findings confirm the method's performance and its alignment with the analytical objectives set by the manufacturer and professional societies. This ensures compliance with both regulatory and normative requirements.

Keywords: Thyroglobulin autoantibodies; Analytical performance; Repeatability; Reproducibility; Alinity CI analyzer; Immuno-chemiluminescence

1. Introduction

The verification and validation of an analytical method involve assessing the performance of the analytical process, which includes parameters such as precision, accuracy, trueness, measurement range, sensitivity to interference, and detection limit when applicable. This process requires quantifying these performance characteristics using a standardized operating protocol [1], then judging them against criteria defined by learned societies (RICOS, FSCB) or the supplier. Moreover, the verification of an analytical performance is both a regulatory requirement outlined in The Moroccan Guide for the good performance of Medical Laboratory Analysis (GBEA) and a normative standard according to ISO 15189:2022 [2][3],

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.

* Corresponding author: Imane Douichi

In this study, we carried out a method verification protocol for the thyroglobulin autoantibodies (TgAb), using Abbott's Alinity ci® automated system. The aim of our work is to carry out a study which forms an essential basis for an accreditation procedure and is part of the quality process to which our laboratory is strongly committed.

1.1. TgAb and dosing interests

Thyroglobulin (TG) is a 660 kDa hyperglycosylated protein expressed in thyrocytes and secreted into the follicular lumen, where it accumulates. Dimeric TG undergoes iodination at specific tyrosine residues in a process influenced by dietary iodine intake. Iodinated TG is transported to the thyrocyte cytosol via pinocytosis and digested, releasing the thyroid hormones triiodothyronine (T3) and thyroxine (T4). TG serves as both a precursor for thyroid hormone biogenesis, which is regulated by thyroid-stimulating hormone (TSH) signaling, and as a carrier protein responsible for iodine storage within the follicular colloid [4] [5].

Thyroglobulin antibodies (TgAb) are predominantly of the IgG class, primarily IgG1, with some IgG2, minimal IgG3, and very low levels of IgG4. TgAb levels tend to fluctuate in parallel with TPOAb (thyroid peroxidase antibody) levels under various clinical conditions, such as during methimazole therapy, after 131I therapy, and during pregnancy or the postpartum period. TgAb are less prevalent than TPOAb in patients with Graves' disease but are present at similar rates in patients with Hashimoto's thyroiditis and in family members of individuals with autoimmune thyroid disease. [6]

TgAb is a crucial marker in the context of thyroid cancer, particularly for detecting residual disease or recurrence. Measuring TgAb levels provides valuable insights into the progression from subclinical thyroid disorders to overt hypothyroidism and enhances our understanding of the immune mechanisms underlying thyroid autoimmunity. [6]

While TgAb levels often mirror those of TPOAb in clinical conditions, TgAb holds particular importance in monitoring thyroid cancer but is generally less sensitive than TPOAb for predicting autoimmune thyroid disease. [6]

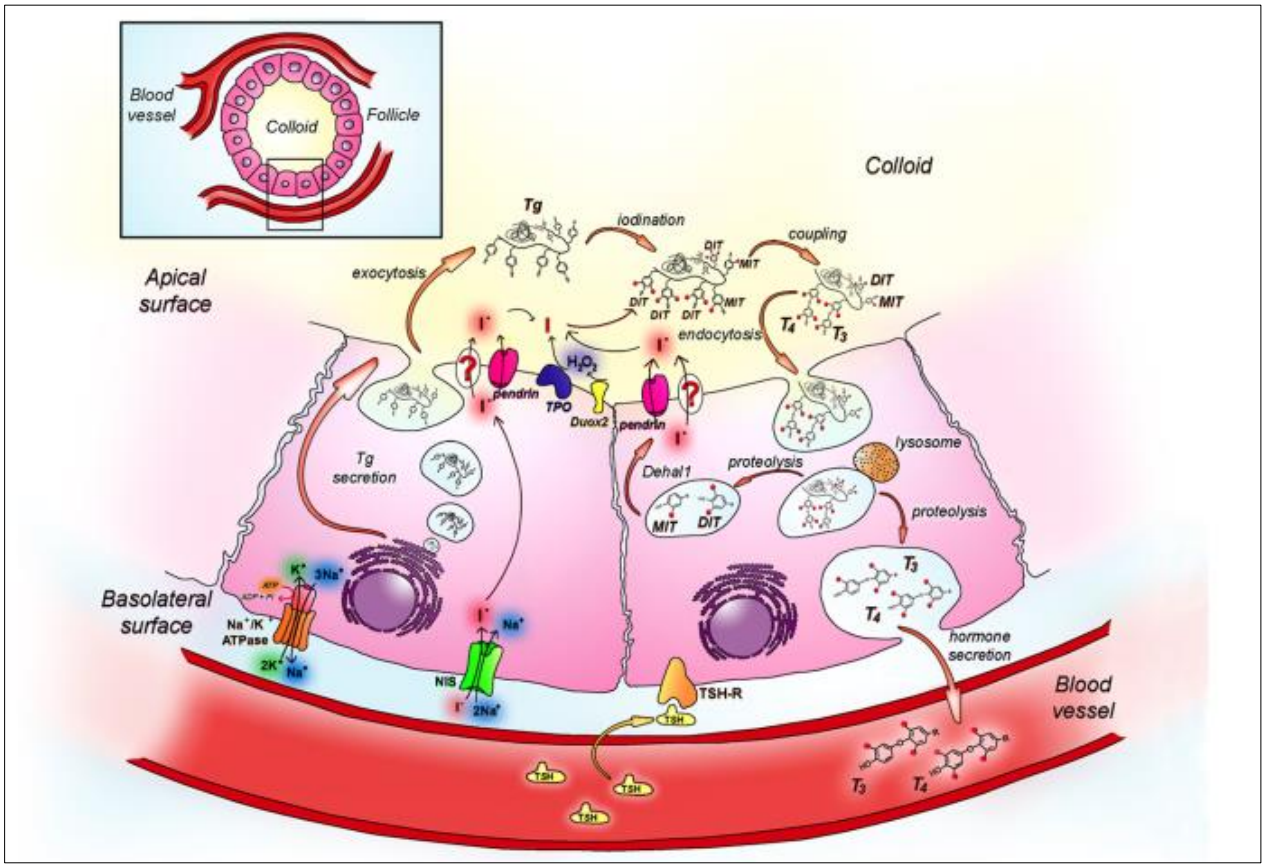

Figure 1 Tg structure and function [5]

1.2. Principle of the assay method

This assay is a two-step immunological test for the quantitative determination of IgG-type autoantibodies directed against thyroglobulin (anti-Tg) in human serum and plasma, utilizing chemiluminescent microparticle immunoassay (CMIA) technology.

The sample, paramagnetic microparticles coated with thyroglobulin, and the assay diluent are combined and incubated. Anti-Tg antibodies present in the sample bind to the thyroglobulin-coated microparticles. After washing, an acridinium-labeled anti-human IgG antibody conjugate is added to form a reaction mixture, which is then incubated. Following another wash cycle, pre-activation and activation solutions are added.

The resulting chemiluminescent reaction is measured in relative light units (RLUs). There is a direct relationship between the amount of anti-Tg antibodies in the sample and the RLUs detected by the optical system.

2. Material and methods

Our study was carried out in the biochemistry laboratory of the Mohammed VI University Hospital in Oujda over a period of 33 days. TgAb was tested for analytical performance on the Abbott Alinity ci automated system, following the reproducibility and repeatability protocol described in the COFRAC GTA 04 accreditation technical guide. In our study, we chose the multi-specific control as the sample to guarantee more reliable and reproducible results. Three sample levels (Low, Medium and High) were used; each tested 33 times to assess repeatability. Then, over a period of 31 days, we assessed reproducibility by running the control daily at the three levels: low, medium and high.

The standard deviation (SD), mean and coefficient of variation (CV) are processed by BYG Informatics EVM statistical software during the evaluation process, and then compared with the standard values of the learned societies (RICOS), no CV reference values were estabilished by Frensh Society of Clinical Biology SFBC. The findings of this investigation are detailed in the sections that follow .

3. Results

3.1. Repeatability Results

Repeatability is evaluated by repeatedly analyzing the same samples under uniform conditions by the same operator. This process considers all aspects of measurement, including reagents, calibration, instrumentation, and operator performance, conducted within the shortest possible timeframe. The repeatability test determines initial system performance and verifies the proper functioning of the instrument-reagent system for the analyte in question [7]. Variability is assessed using coefficient of variation (CV) values. As shown in Table 1, the results for the various TgAb assay verification criteria indicate satisfactory repeatability at three level low, medium and high with CV values of 3.13%, 2.90 % and 2.40%, respectively.

These findings have been graphically presented using Levey-Jennings plots to further illustrate the obtained results (Figures 2,3 and 4).

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

Levels	N	Mean	Standard deviation (SD)	CV %	CV% (RICOS)
LOW	33	16.71 UI/ml	0.523 UI/ml	3.13 %	3.19 %
MEDIUM	33	33.94 UI/ml	0.985 UI/ml	2.90 %	3.19 %
HIGH	33	44.31 UI/ml	1.066 UI/ml	2.40 %	3.19 %

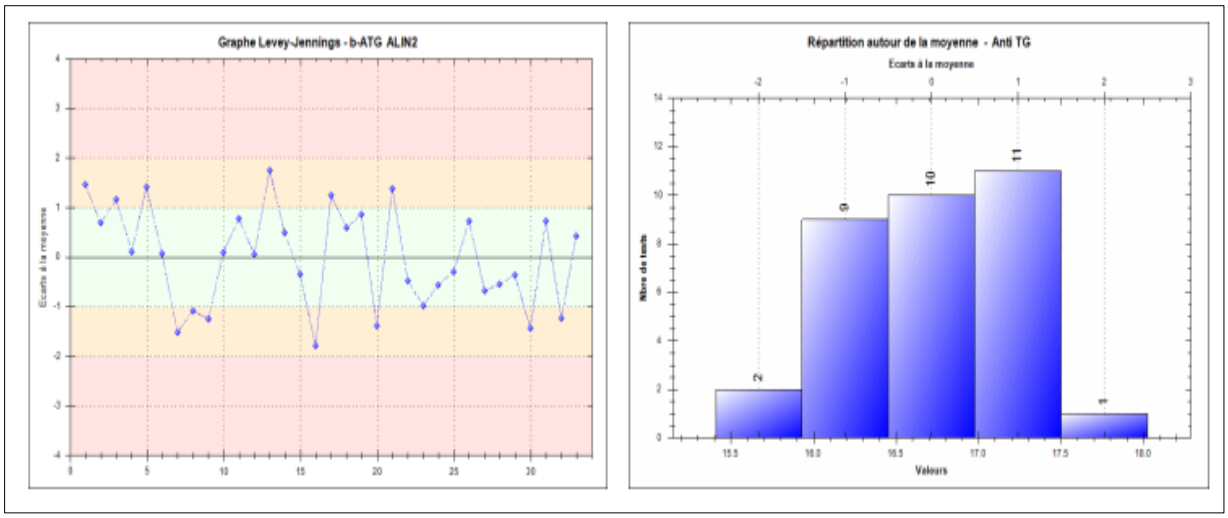

Figure 2 Low Level of Repeatability of TgAb: Levey Jennings graph and the distribution around the mean

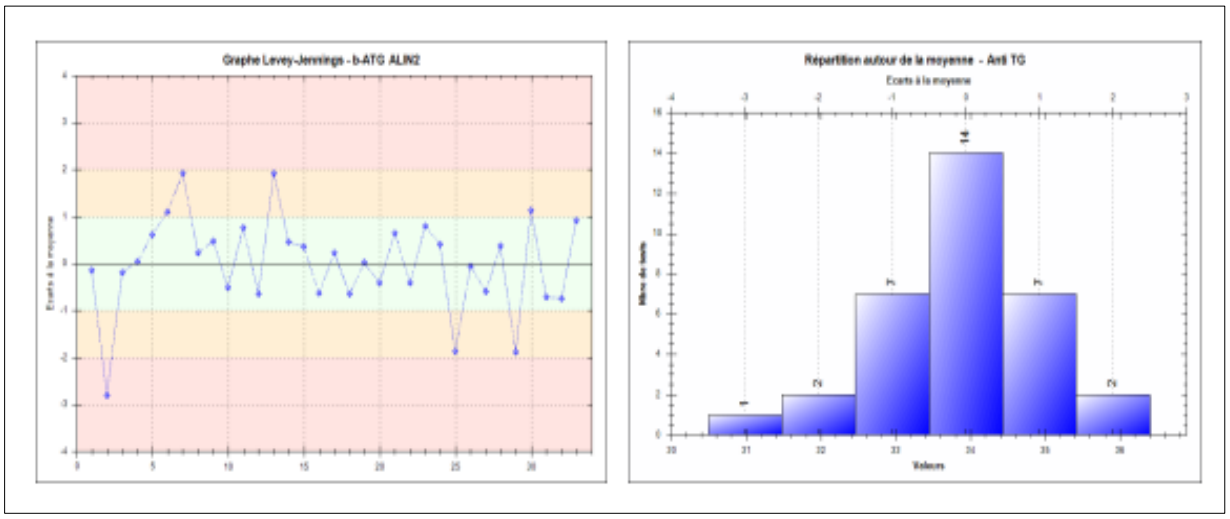

Figure 3 Medium Level of Repeatability of TgAb: Levey Jennings graph and the distribution around the mean

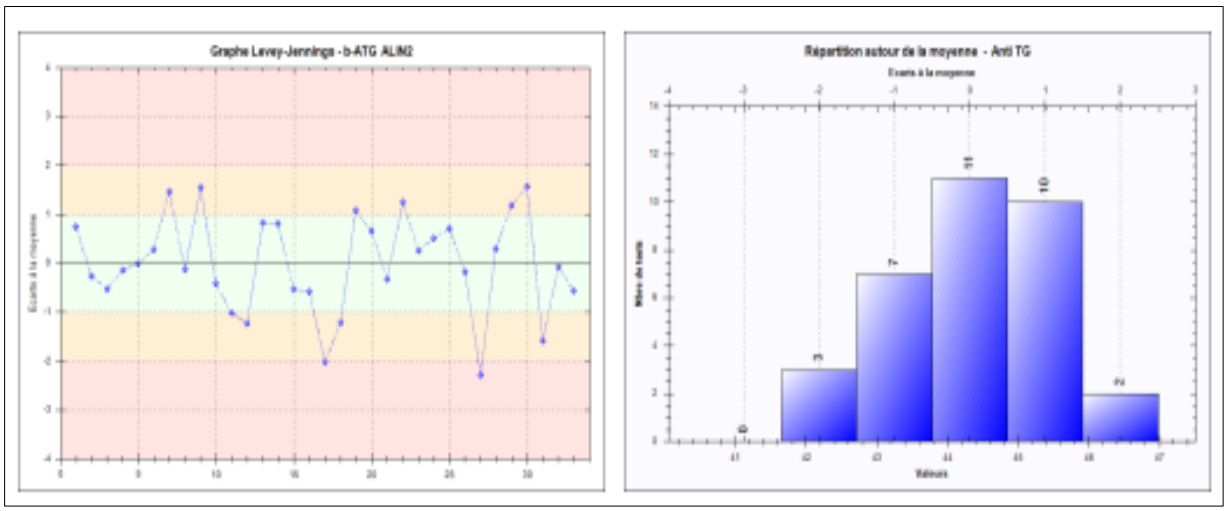

Figure 4 High Level of Repeatability of TgAb : Levey Jennings graph and the distribution around the mean

3.2. Reproducibility results

The reproducibility test involves analyzing the same sample under varying conditions to evaluate the impact of factors such as operators, time, reagent batches, and calibrations on the results. This test aims to define acceptance criteria, particularly for decision support systems. Variability is quantified using the Coefficient of Variation (CV). [7]

For the low, medium, and high levels, the CV values are provided (CV1 = 4.25 %, CV2 = 5.12%, CV3 = 5.13 %) These results are illustrated on the Levey-Jennings graphs (Fig. 5,6 and 7fig).

Table 1 Reproducibility results for TgAb on the Alinity i® automated system by level with comparison to RICOS data

Levels	N	Mean	Standard deviation (SD)	CV %	CV% (RICOS)
LOW	31	30.56 UI/ml	0.781 UI/ml	2.56 %	4.25 %
MEDIUM	31	90.30 UI/ml	3.253 UI/ml	3.60 %	4.25 %
HIGH	31	199.44 UI/ml	5.391 UI/ml	2.70 %	4.25 %

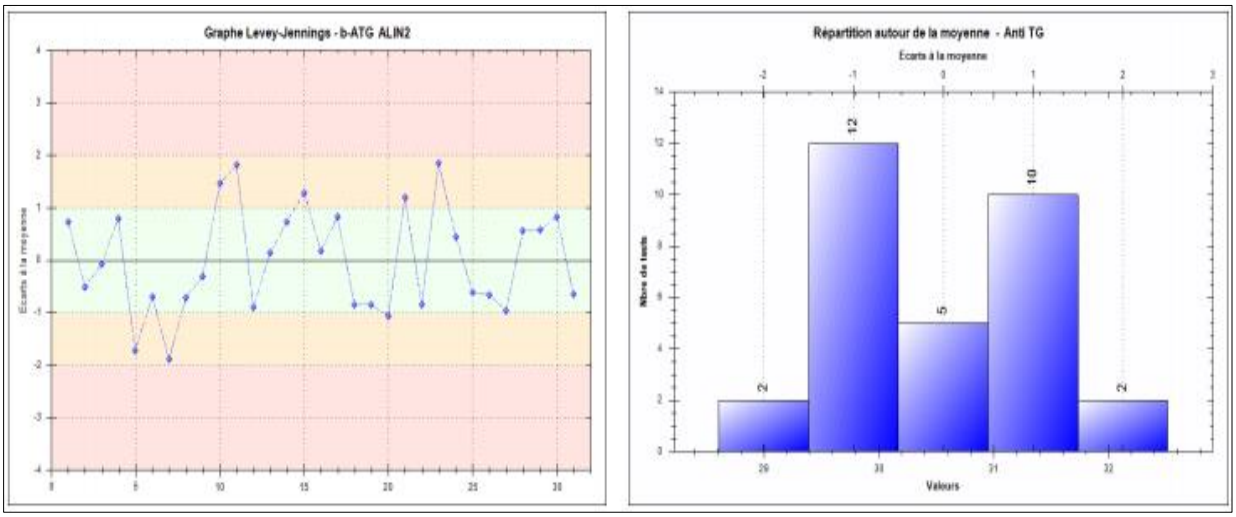

Figure 5 Low level of reproducibility of TgAb : Levey Jennings graph and distribution around the mean

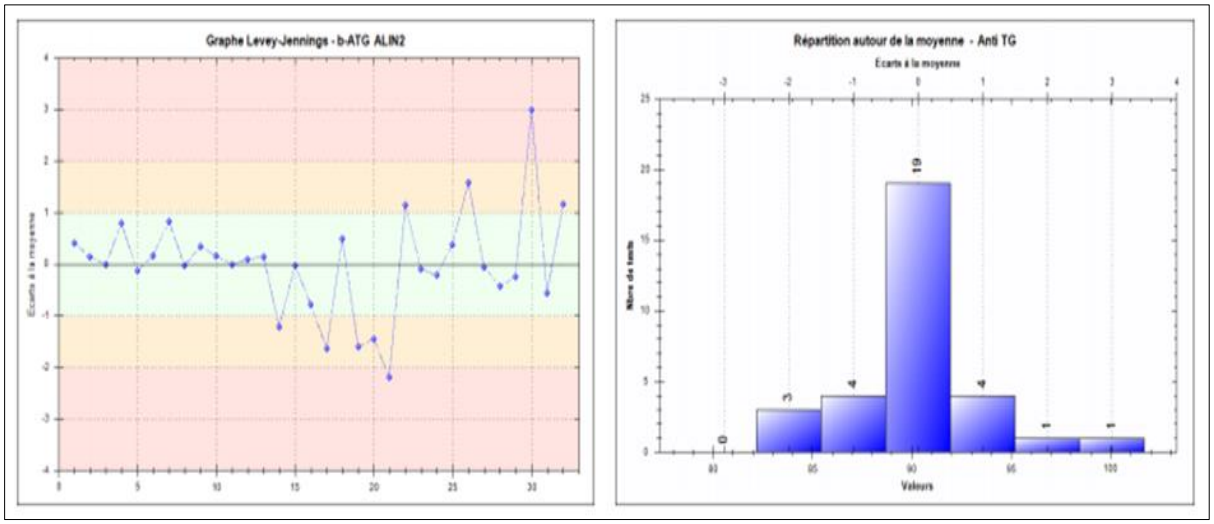

Figure 6 Medium level of reproducibility of TgAb : Levey Jennings graph and distribution around the mean

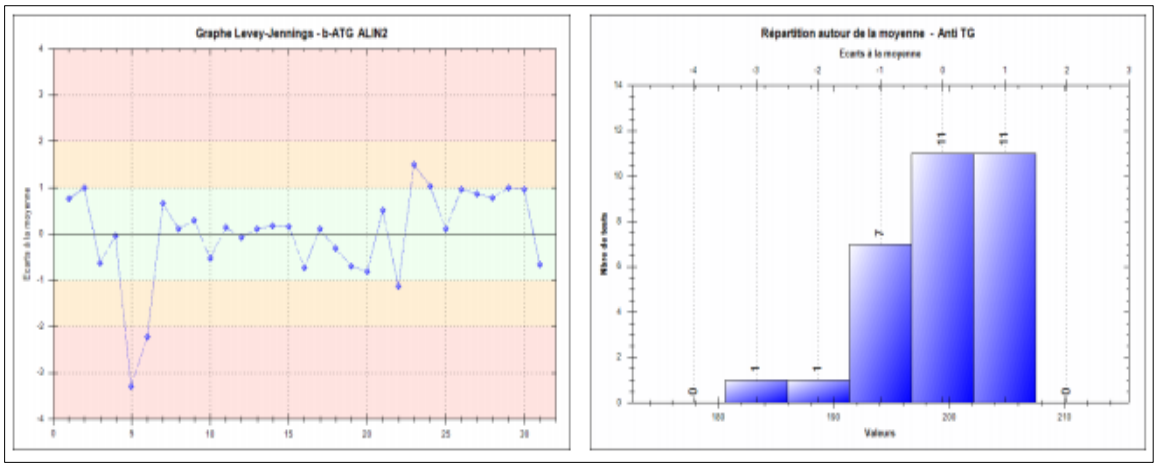

Figure 7 High level of reproducibility of TgAb : Levey Jennings graph and distribution around the mean

4. Discussion

Autoimmune thyroiditis was first identified by Hashimoto in 1912. [8]This form of autoimmunes thyroid disease, characterized by the presence of a goiter, is known as Hashimoto's thyroiditis. In 1956, Roitt et al. [9] were the first to demonstrate the existence of thyroglobulin autoantibodies (TgAb) in patients with this condition, using a precipitin reaction. Unlike TPOAb (thyroid peroxidase antibody), anti-Tg antibodies are not considered pathogenic but rather serve as markers of the disease [10]. These antibodies have been shown to be polyclonal and exhibit heterogeneity in their heavy chain subclasses. [11] [12]

Thyroglobulin is a 670,000 Dalton glycoprotein composed of two identical subunits, making it the primary protein in the thyroid gland. It provides 40 of its 140 tyrosine residues for iodination, playing a crucial role in the biosynthesis of thyroxine (T4) and triiodothyronine (T3), and is therefore essential for iodine storage within the thyroid gland. [13]

While anti-Tg antibodies commonly occur alongside anti-TPO antibodies in the majority of cases of Hashimoto's thyroiditis, primary myxedema, and Graves' disease [14] [15], they are the sole antibodies present in up to 1% of hypothyroidism cases [16]. These antibodies are associated with mild forms of hypothyroidism or hyperthyroidism and are frequently detected in individuals with other autoimmune conditions, such as rheumatoid arthritis, pernicious anemia, and type I diabetes [17] [18].

Anti-Tg antibodies are found in 30% to 60% of patients with thyroid carcinoma. In these cases, the measurement of thyroglobulin antigen must account for the potential presence of significant concentrations of anti-Tg antibodies, as their presence can affect the accuracy of antigen detection and quantification [19] [20]. Additionally, low levels of anti-Tg antibodies are identified in about 20% of asymptomatic individuals, particularly among the elderly and more frequently in women than men, though the clinical relevance of these autoantibodies remains uncertain [21] [22].

The Abbott Alinity ci is a multiparametric system capable of integrating clinical chemistry and immunoassay, enabling the measurement of a wide range of standard biochemical parameters as well as specific proteins.

The CMIA (microparticle chemiluminescence immunoassay) method is already being utilized for the TgAb assay. As a result, validation is not necessary; instead, we only need to conduct verification according to a "scope A verification/validation" where the recognized methods, are pre-validated within their designated field of application, to ensure the accuracy and the reliability of our results [3].

This verification is essential, meeting both regulatory standards (as per the Moroccan Guide for the Proper Execution of Medical Laboratory Analyses GBEA and normative requirements (ISO 15189:2022). Setting predetermined analytical goals through this control ensures the production of precise and dependable results [23].

The reproducibility test is employed to assess the consistency of assay results when different variables are introduced. [23]Our study results affirmed the reliability of the TgAb assay for reproducibility assessment. The three levels low, medium and high yielded satisfactory outcomes. For each level, 31 values were analyzed, revealing means of m1 = 0.74 IU/ml and m2 = 25.19 IU/ml, along with coefficients of variation (CV) of CV1 = 2.56 %,

CV2 =3.60% and CV3= 2.70 %. The low CV values signify that even when modifying various factors, the test consistently produces results close to the mean value. This reliability is crucial in medical testing, where consistency ensures the dependability of test results for clinical decisions. The fact that CV values align with established quality control limits indicates that the test adheres to industry standards for reproducibility, enhancing its suitability for precise diagnostic applications.

The precision of the assay under regulated and ideal circumstances is the main emphasis of the repeatability test. This evaluation is crucial as it gauges the method's capability to produce consistent results when analyzing the same sample repeatedly.

In examining the repeatability across two levels (low and high), 30 values were scrutinized for each level, revealing remarkably low coefficients of variation (CV): CV1 = 3.13 %,

CV2 = 2.90 % and CV3 = 2.40%. These values indicate a small degree of variability, underscoring the high precision of the assay.

The extremely low CV values highlight the assay's outcomes as highly stable and predictable when operating under controlled conditions. Such precision is of utmost importance in clinical testing, where even minor variations can carry significant implications for patient care.

The Mohammed VI University Hospital's central laboratory in Oujda has implemented a quality strategy incorporating a method verification protocol. Conducting this type of investigation will enable the establishment of a credible accreditation process for the analyses conducted in our laboratory [24].

As a pivotal reference center in the Eastern region of Morocco, our laboratory serves not only the needs of referred or hospitalized patients but also contributes to assessing the overall health of the region's general population through various scientific studies [25].

5. Conclusion

The verification of the TgAb assay demonstrated robust analytical performance, with excellent precision and reproducibility across all tested levels. These findings establish the assay's reliability for routine clinical applications, particularly in diagnosing and monitoring of autoimmune thyroid disorders and differentiated thyroid cancer (DTC).

By meeting ISO 15189 and other regulatory standards like RICOS standards, the Mohammed VI University Hospital's central laboratory underscores its dedication to delivering accurate diagnostic results, enhancing both patient care and regional health outcomes.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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