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Verification of the analytical performance of the serum anti-thyroperoxidase antibody assay on the Abbott Alinity ci®: Experience from the Biochemistry Laboratory of the Mohammed VI University Hospital of Oujda

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Abstract

The objective of this study was to evaluate the analytical performance of the measurement of anti-thyroperoxidase antibodies (anti-TPO antibodies) in human serum and plasma, using chemiluminescent microparticle immunoassay (CMIA), in accordance with Domain A criteria of the medical biology method verification/validation guide.

The results of the anti-TPO antibody serum assays show good repeatability for the three levels (1: low, 2: medium, and 3: high), with respective coefficients of variation (CV) of 1.67%, 1.97%, and 1.17%. Similarly, intermediate precision is considered satisfactory, with CV of 1.90% for level 1, 1.96% for level 2, and 1.38% for level 3. Comparison with the reference CV defined by the RICOS (Reference Institute for Clinical and Healthcare Standards) confirms the reliability and compliance of the results.

The results allowed us to verify the performance of the light chain Lambda assay method and compare it with the analytical objectives set in the accreditation process in which our laboratory is engaged.

Keywords: Anti-thyroperoxidase antibody assay; Analytical performance; Repeatability; Reproducibility; Coefficient of variation; Alinity ci® analyzer

1. Introduction

The measurement of anti-thyroperoxidase (anti-TPO) antibodies in serum is a diagnostic procedure used to assess their concentration in a blood sample. It is one of the key tests for detecting anti-thyroid autoantibodies, essential for diagnosing autoimmune thyroid diseases (1). The anti-TPO antibody is one of the parameters measured in our laboratory using the automated Abbott Alinity ci® system. The verification of the anti-TPO antibodies determination method is critical to ensure the quality of our results.

Analytical method verification in the laboratory involves assessing method performance by following a standardized protocol. This protocol is based on criteria established by reference organizations, such as the Reference Institute for Clinical and Healthcare Standards (RICOS) and the French Society of Clinical Biology (SFBC) (2). This process ensures that the laboratory maintains the quality and reliability of the method, verifies its performance, and confirms that the results are robust enough to provide reliable and accurate clinical interpretations, essential for both the patient and the prescriber.

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The aim of our study is to evaluate the analytical performance of the anti-TPO antibodies test using chemiluminescent microparticle immunoassay (CMIA), in accordance with the criteria outlined in Domain A of the medical biology method verification/validation guide.

1.1. Reminder of anti-TPO antibodies

It was first demonstrated by Trotter et al. in 1957 and later by Roitt and Doniach in 1958, that many patients with Hashimoto's thyroiditis had detectable autoantibodies in their blood, directed against a thyroid antigen distinct from thyroglobulin. This antigen was called the "microsomal thyroid antigen," and it has since been established that the majority of thyroid anti-microsomal antibodies recognize thyroid peroxidase (TPO) (3).

TPO is a membrane-bound glycoprotein enzyme with an approximate mass of 107 kDa. Its in vivo function is the iodination of tyrosine in the synthesis of thyroid hormones T3 and T4. The autoimmune reactivity directed against TPO is considered polyclonal and heterogeneous, involving at least six recognized antigenic determinants, including both conformational and linear epitopes. Additionally, the proportion of each immunoglobulin class (IgG or IgM) or subclass (IgG1 - IgG4), as well as their affinity, varies widely from one patient to another. Unlike autoantibodies directed against thyroglobulin (anti-Tg), anti-TPO antibodies fix complement, are potentially deleterious, and could play a pathogenic role in autoimmune thyroid diseases with a destructive nature (3).

Anti-TPO antibodies are frequently found in association with anti-Tg antibodies in most cases of Hashimoto's thyroiditis, primary myxedema, and Graves' disease. They are also detectable in the majority of postpartum thyroiditis cases, and their presence early in pregnancy is associated with an increased risk of asymptomatic postpartum hypothyroidism. In some cases, anti-TPO antibodies are found in the absence of anti-thyroglobulin antibodies, particularly in patients with small goiters. Approximately 64% of autoimmune hypothyroidism cases are exclusively associated with anti-TPO antibodies. Additionally, these antibodies are often found in patients with other autoimmune diseases, such as rheumatoid arthritis, Addison's disease, and type 1 diabetes. They may also be detected at low levels in up to 20% of asymptomatic individuals, especially among the elderly, with a higher prevalence in women. However, the clinical significance of these autoantibodies remains uncertain (3).

1.2. Principle of the assay method

The assay is a two-step immunological test used for the quantitative determination of anti-TPO antibodies in human serum and plasma, utilizing the chemiluminescent microparticle immunoassay (3).

First, the sample, paramagnetic microparticles coated with TPO, and the assay diluent are combined and incubated, allowing the anti-TPO antibodies in the sample to bind to the TPO-coated microparticles. The mixture is then washed to remove unbound elements. A conjugate of human anti-IgG antibodies labeled with acridinium is added to the reaction mixture, followed by another incubation. After a second washing cycle, pre-trigger and trigger solutions are added, inducing a chemiluminescent reaction. The intensity of this reaction is measured in relative light units (RLU) (3).

There is a direct relationship between the concentration of anti-TPO antibodies in the sample and the light units detected by the optical sensors of the system.

2. Material and methods

This prospective study was conducted at the biochemistry laboratory of Mohammed VI University Hospital over a period of 30 days. It primarily focused on evaluating the reproducibility and repeatability of the anti-TPO antibody assay.

The study was carried out in two phases. In the first phase, daily control measurements of anti-TPO antibodies were performed at three levels—low, medium, and high—over 30 days to assess reproducibility and ensure the consistency of the results. A set of serum samples, evenly distributed according to their anti-TPO antibody concentration, was then classified into three groups (low, medium, and high) for analysis.

In the second phase, serum samples with a wide range of anti-TPO antibody concentrations were collected, covering the entire measurement spectrum. These samples were grouped into three categories: low, medium, and high. For each sample, 30 replicates were performed to assess repeatability. The analysis was carried out using the Alinity ci® anti-TPO antibody reagent kit on the immunoassay system.

We followed an operational approach according to the COFRAC accreditation technical guide GTA 04. Data processing and statistical analysis were conducted using the intermediate module EVM, provided by the BYG middleware, which acts as a gateway between Alinity ci® and the iLab result validation software. The Coefficient of Variation (CV) values in our study were compared to those defined by the RICOS standards organization.

The results of this analysis are presented in the following sections.

3. Results

3.1. Reproducibility results

The intermediate precision test, or intra-laboratory reproducibility, involves analyzing the same sample under varying conditions to assess the influence of different factors (operator, reagents, calibrations) on the accuracy and reliability of the test. This approach helps establish acceptance criteria by considering biological variations, thereby optimizing diagnostic methodologies and improving the quality of laboratory analyses.

The collected data allow for the calculation of the mean, standard deviation, and CV for each series, between series, within series, and for the entire dataset.

The intermediate precision results were satisfactory for all three levels—low, medium, and high—with respective CV of 1.90%, 1.96%, and 1.38%. These values fall within the limits defined by RICOS and are compared to the predefined thresholds, as shown in Table 1. For better visualization, they are illustrated in Levey-Jennings charts (Fig. 1, Fig. 2, Fig. 3).

Table 1 Reproducibility results for anti-TPO antibodies on the Alinity ci® automated system by level with comparison to RICOS data (with expansion of coefficient k = 1,211)

Level of IQC	Number of Values	Mean (UI/ml)	Standard Deviation	Coefficient of Variation CV (%)	Reference CV: RICOS (%)
Low	30	17.88	0.339	1,90 %	5.65 %
Medium	30	29.66	0.583	1,96 %	5.65 %
High	30	37.88	0.523	1,38 %	5.65 %

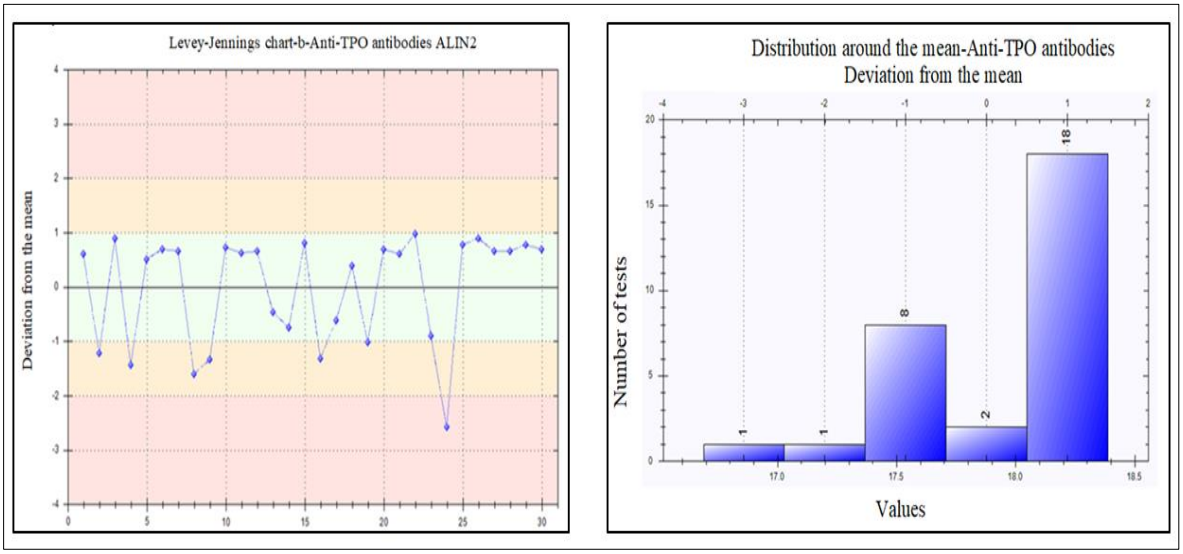

Figure 1 Low Level of Reproducibility: Levey Jennings graph and the distribution around the mean –Anti-TPO antibodies

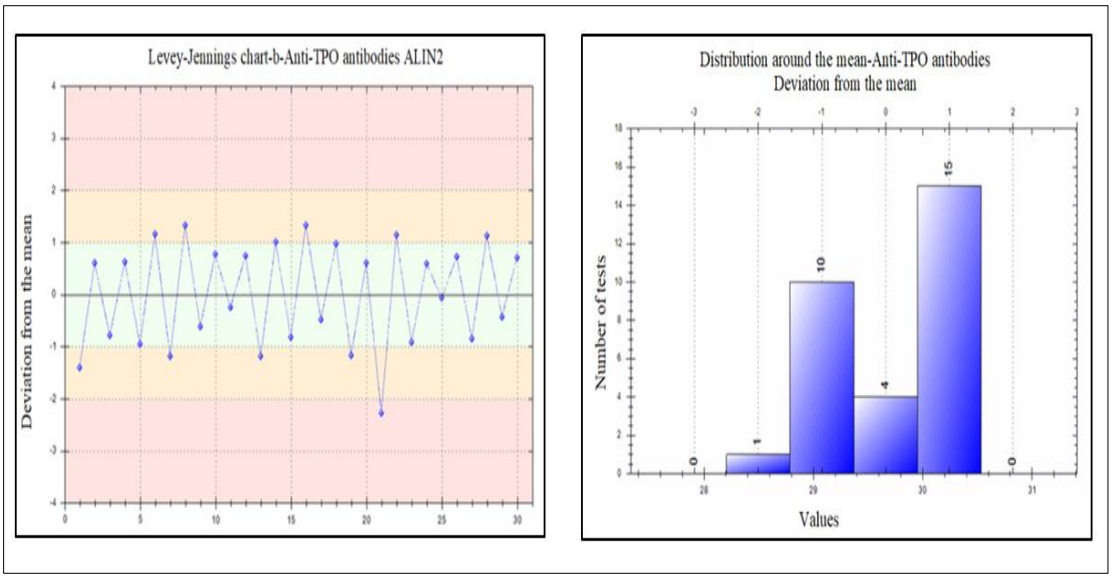

Figure 2 Medium Level of Reproducibility: Levey Jennings graph and the distribution around the mean –Anti-TPO antibodies.

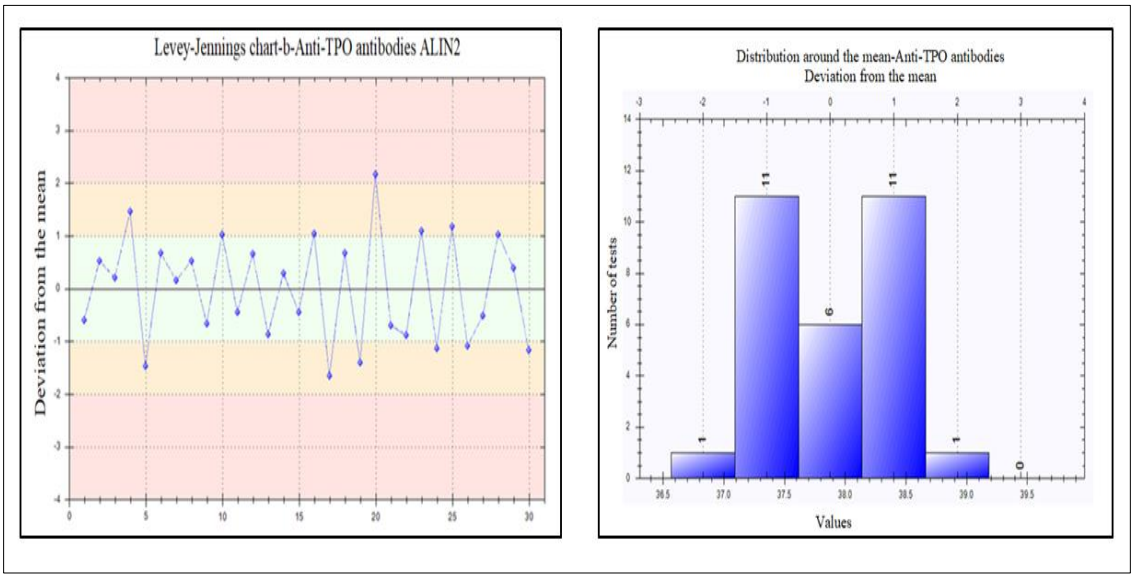

Figure 3 High Level of Reproducibility: Levey Jennings graph and the distribution around the mean –Anti-TPO antibodies

3.2. Repeatability results

Repeatability is assessed by repeatedly analyzing the same sample under identical conditions (operator, reagent, calibration, and instrument) to determine the system's initial performance and verify its proper functioning for the studied analyte.

The repeatability of measurements was evaluated using the CV. The results of the anti-TPO antibody assay demonstrate satisfactory repeatability across the three levels—low, medium, and high—with respective CV values of CV1 = 1.67%, CV2 = 1.97%, and CV3 = 1.17% over 30 samples. These results are graphically represented in the Levey-Jennings charts (Fig. 4, Fig. 5, Fig. 6).

The conclusion for each level confirms that the repeatability CV is reliable and remains below the acceptable limit. Similar to the intermediate precision results, the thresholds defined by RICOS, which incorporate expansion factors, are considered. The obtained CV values are then compared to these reference thresholds (Table 2).

Table 2 Repeatability results for Anti-TPO antibodies on the Alinity ci® automated system by level with comparison to RICOS data (with expansion of coefficient k = 1,211)

Level of IQC	Number of Values	Mean (UI/ml)	Standard Deviation	Coefficient of Variation CV (%)	Reference CV: RICOS (%)
Low	30	17.91	0.298	1,67 %	4.24%
Medium	30	29.62	0.583	1,97 %	4.24%
High	30	37.88	0.442	1,17 %	4.24%

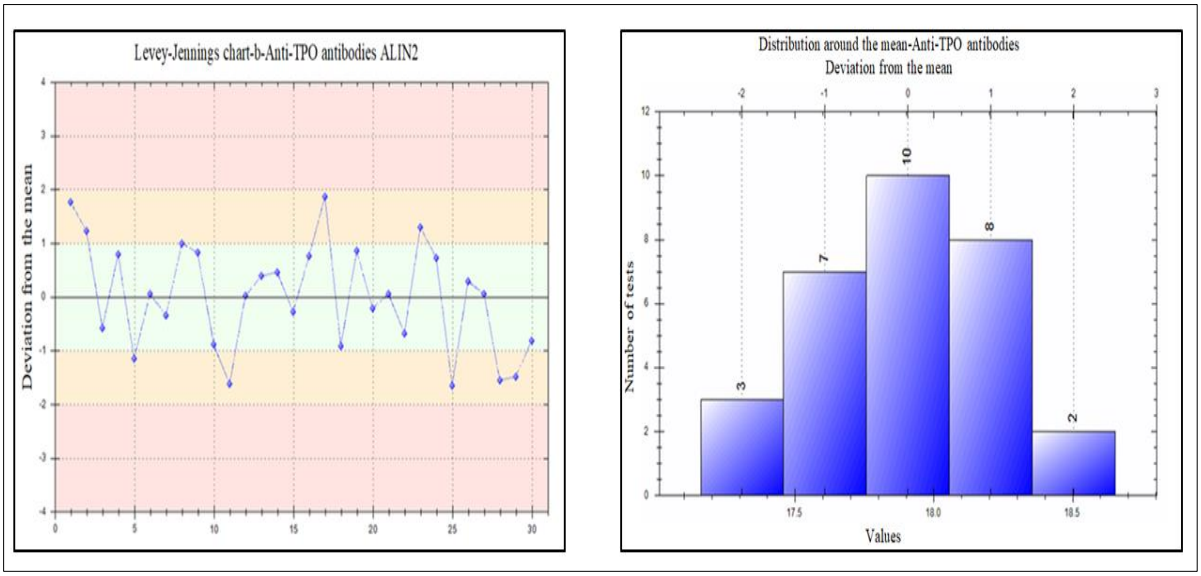

Figure 4 Low Level of Repeatability: Levey Jennings graph and the distribution around the mean –Anti-TPO antibodies

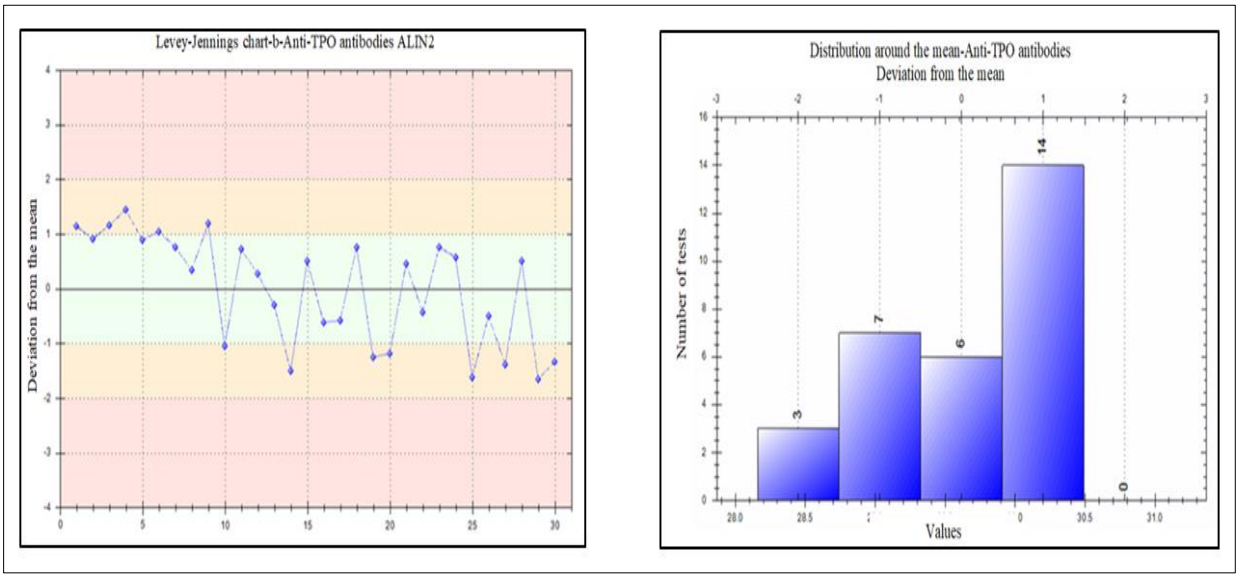

Figure 5 Medium Level of Repeatability: Levey Jennings graph and the distribution around the mean –Anti-TPO antibodies

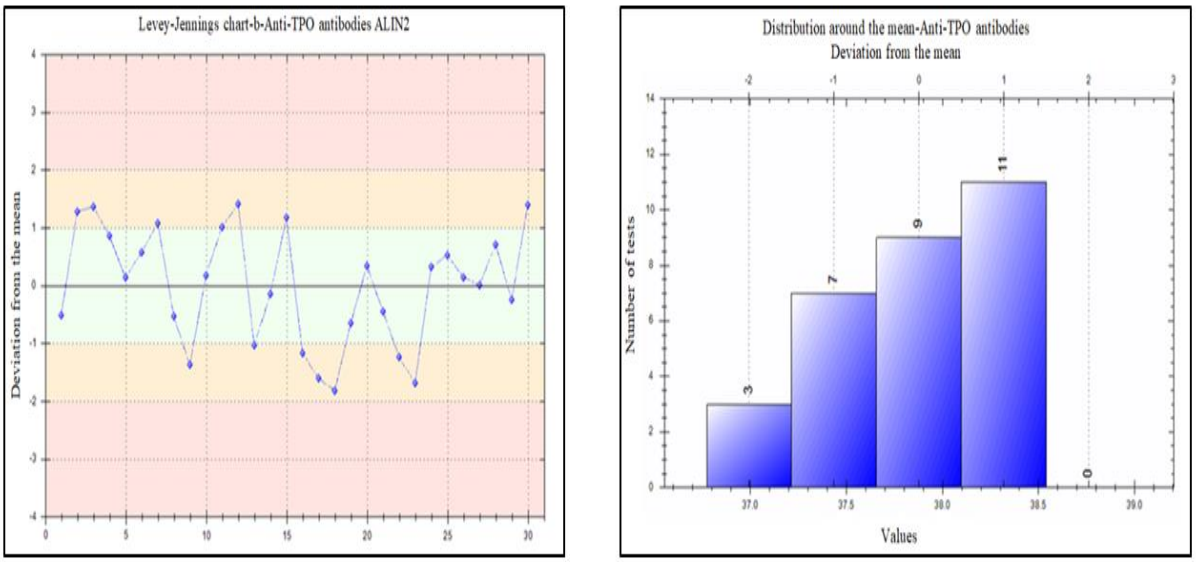

Figure 6 High Level of Repeatability: Levey Jennings graph and the distribution around the mean –Anti-TPO antibodies

4. Discussion

Thyroid peroxidase (TPO) is a membrane-bound enzyme essential for thyroid hormone synthesis. Located at the apical pole of thyroid cells, it catalyzes the iodination and coupling of tyrosyl residues in thyroglobulin. Anti-TPO antibodies are autoantibodies directed against this enzyme and serve as a sensitive and specific marker for autoimmune thyroid diseases, particularly Hashimoto's thyroiditis and Graves' disease. They are detected in 90–98% of Hashimoto's thyroiditis cases and 70–85% of Graves' disease cases. Their measurement is therefore crucial for the diagnosis and monitoring of these conditions (1). Anti-TPO antibody levels are significantly elevated in affected patients, allowing for the identification of thyroid disorders (4). The measurement method for anti-TPO antibodies offers enhanced sensitivity, accuracy, and specificity, enabling better tracking of fluctuations in antibody levels over time. This makes it an essential tool in the clinical monitoring of autoimmune thyroid diseases (5).

Anti-TPO antibody detection tests are highly sensitive and accurate. They rely on the interaction between autoantibodies and an iodine-125-labeled antigen, allowing for the measurement of very high antibody concentrations. These tests show strong correlation with other methods but are more sensitive and do not exhibit cross-reactivity with anti-thyroglobulin antibodies. They provide a reliable method for studying antithyroid autoantibodies (6).

The detection methods for anti-TPO antibodies have evolved from radioimmunoassay to luminescent immunoassay, enabling automation, increased sensitivity, faster analysis, and the elimination of radioactive contamination risks. Based on acridinium ester labeling, this technology is utilized in Abbott's direct chemiluminescence systems.

To ensure the reliability of the obtained results, a verification procedure is essential. This process involves assessing repeatability, reproducibility, linearity, sensitivity, and specificity to evaluate the laboratory's overall analytical performance. These assessments are conducted in accordance with quality standards such as those defined by ISO 15189 (7,8).

The reproducibility test assesses the consistency of results by considering variables such as operators, time, reagent batches, and calibrations. This variability is measured by the CV, which reflects the deviation of results from the mean. The CV values for low, medium, and high levels are 1.90%, 1.96%, and 1.38%, respectively, indicating low dispersion and good consistency of results. These findings confirm that the chemiluminescent microparticle immunoassay method is stable and reliable under various conditions.

Even in the presence of variations, such as those related to operators or reagent batches, the low CV values indicate that the test produces consistent results close to the mean. This stability is crucial in the medical field, where result reliability

is essential for making safe clinical decisions. Furthermore, compliance with quality control limits confirms that the test meets industry reproducibility standards, reinforcing its utility as a precise diagnostic tool.

The repeatability test evaluates the precision of the assay under optimal conditions by measuring result consistency when the same sample is analyzed multiple times. The CV values obtained for repeatability are remarkably low: CV1 = 1.67%, CV2 = 1.97% and CV3 = 1.17%, indicating minimal variability and confirming the high precision of the test. These results demonstrate that the chemiluminescent microparticle immunoassay method ensures consistent and accurate measurements, even with repeated analyses of the same sample.

Exceptionally low CV values indicate that test results are highly stable and predictable under controlled conditions. This precision is critical in clinical testing, where even small variations can impact patient care decisions. The alignment of CV values with quality control standards further strengthens the reliability of the test and confirms its ability to produce reproducible results.

At Mohammed VI University Hospital in Oujda, the central laboratory has implemented rigorous method verification protocols to uphold high standards in patient care and laboratory services (9,10). This quality-focused approach ensures the accuracy of anti-TPO antibody testing, a key indicator for diagnosing autoimmune thyroid diseases, thereby contributing to optimal diagnostic and therapeutic outcomes. The study evaluated the reproducibility and repeatability of anti-TPO antibody quantification, showing low CV values in compliance with RICOS standards. These results confirm the reliability, sensitivity, and precision of the test, reinforcing its effectiveness for clinical use and enhancing the quality of diagnostic laboratory analyses.

5. Conclusion

Anti-TPO antibodies are key biomarkers in the diagnosis and monitoring of autoimmune thyroid diseases. Performance evaluation has shown that the Alinity ci® analyzer, an automated immunoassay system, offers excellent detection capabilities for anti-TPO, meeting the requirements of daily clinical diagnostics. Thanks to its direct chemiluminescence method, it demonstrates high resistance to interferences, although it is limited by relatively short luminescence duration.

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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