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

(RESEARCH ARTICLE)

Check for updates

Verification of the analytical performance of the Human thyroid stimulating hormone assay on ALINITY ci ® Experience from the biochemistry laboratory of Mohammed VI University Hospital in Oujda

KIDOUN Asmae [1, 2, *], KRIMI Kholoud [1, 2], DOUICHI Imane [1, 2], El Moujtahide Dounia [1, 2], El Houcine Sebbar [1, 2] and Mohammed Choukri [1, 2]

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

World Journal of Biology Pharmacy and Health Sciences, 2025, 21(03), 519-526

Publication history: Received on 26 June 2024; revised on 08 August 2024; accepted on 10 August 2024

Article DOI: https://doi.org/10.30574/wjbphs.2025.21.3.0499

Abstract

The aim of our work was to evaluate the analytical performance of the Human Thyroid Stimulating Hormone (TSH) determination by a two-step immunoassay using microparticle chemiluminescence immunoassay (CMIA) technology, in accordance with the Scope A criteria of the guide of the verification/validation of medical biology methods.

We evaluated the repeatability and the intermediate precision of the assay. The results obtained are very satisfactory for the three levels (low, medium and high), both for intermediate fidelity, with coefficients of variation (CV) of 5.38%, 4.16%, and 5.81%. Respectively, and for repeatability, with coefficients of variation of CV1 = 2.17%, CV2 = 1.54%, CV3 = 1.98% respectively.

The results obtained made it possible to verify the method's performance and compare it with the analytical objectives set in order to meet the regulatory and normative requirements set by the supplier and learned societies.

Keywords: Human Thyroid Stimulating Hormone; Analytical performance; Repeatability; Reproducibility; Alinity CI analyzer; Immuno-chemiluminescence

1. Introduction

Analytical method verification is a process involving the evaluation of the performance of an analytical method. Its quantification following a standardized operating protocol, then its evaluation against standards established by learned societies (RICOS, FSCB), enables laboratories to acquire in-depth knowledge of their analytical methods, their performance and their limitations, in order to ensure the accuracy of analytical results useful to patients and prescribers. It is imperative to guarantee that these performances are adequate (1) (2)

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.

Thyroid-stimulating hormone (TSH) protein in the human serum is considered as a most sensitive indicator for the diagnosis of several diseases related to hyperthyroidism and hypothyroidism.

* Corresponding author: KIDOUN Asmae.

In this study, we carried out a method verification protocol for TSH, 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. Reminder on TSH

Thyroid-stimulating hormone (TSH) is an a/b heterodimeric synthesized by thyrotroph cells in anterior pituitary gland and released to the circulation in a pulsatile manner (3). The hypothalamus which links the nervous system to the endocrine system via the pituitary gland initially releases thyrotropin releasing hormone (TrH) which in turn stimulate the release of TSH (4). TSH then induces the productions of thyroxine (T4) which influences the amount of triiodothyronine (T3) produced by the thyroid gland (Fig. 1). Physiological functioning of TSH includes secretion and ejection of iodothyronines from the gland, promotion of thyroid growth and stimulation of differentiated thyroid functions (5). This hormone is liable to regulate the body's metabolism including body temperature, weight, heart rate and cholesterol and its maintenance is highly vital for the nervous, skeletal, reproductive tissue function (6). This also plays a major role in the protection of thyroid cells from apoptosis (programmed cell death) and development of an individual organism (ontogeny).

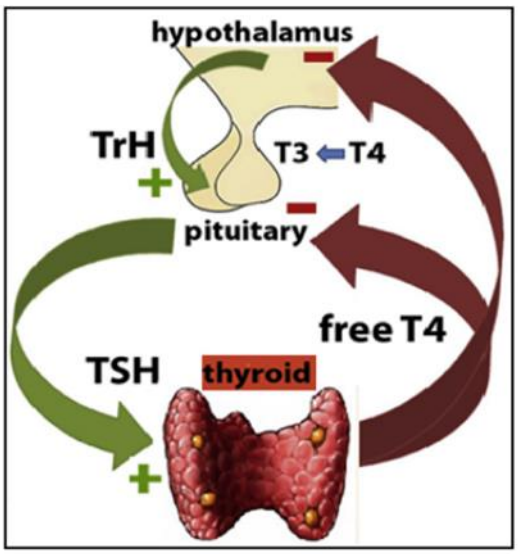

Figure 1 Schematic functioning of thyroid gland (12)

1.2. Principle of the assay method

The assay is a two-step immunoassay using microparticle chemiluminescence immunoassay (CMIA) technology.

In the first step, sample, anti-β TSH antibody coated paramagnetic microparticles and TSH Assay Diluent are combined. TSH present in the sample binds to the anti-TSH antibody coated microparticles. After washing, anti-α TSH acridinium labeled conjugate is added in the second step. Pre-Trigger and Trigger Solutions are then added to the reaction mixture; the resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of TSH in the sample and the RLUs detected by the optical system

2. Material and methods

This study is a prospective investigation conducted within the biochemistry laboratory of Mohammed VI University Hospital, spanning a duration of 30 days. The working methodology adapted is based on the recommendations of the protocol of the French accreditation committee (COFRAC) accreditation technical guide GTA 04. It was structured around two distinct phases. The initial phase involved evaluating the reproducibility of results. This was achieved through daily testing of control samples at three concentration levels—low, medium, and high—over the course of 30 days. The primary aim was to assess the consistency and reliability of the assay. In the subsequent phase, a comprehensive collection of serum samples was amassed, ensuring an equitable distribution of TSH values across the full measurement spectrum. These collected samples were categorized into three groups representing low, medium, and high TSH levels. To gauge repeatability, each serum sample underwent 30 individual assay runs.

The TSH determination was conducted utilizing a dedicated reagent kit on the immunology module of Abbott Alinity CI analyzer. Subsequent data processing was carried out via the BYG middleware, serving as an intermediary software bridging the gap between the Alinity platform and the iLab result validation software. The coefficient of variation (CV) values yielded by this study were subsequently juxtaposed against the standards stipulated by established learned societies, namely the Federation of Clinical Chemistry and Laboratory Medicine (FSCB) and the Reference Institute for Bioanalytics (RICOS).

3. Results

3.1. Reproducibility results

The intermediate fidelity test, also known as intra-laboratory reproducibility, involves analyzing the same sample under diverse conditions, where at least one variable is altered, such as the operator, time, reagent batches, or calibrations. This approach facilitates the establishment of acceptance criteria based on prior knowledge, taking into account biological variations, especially in the context of decision support systems. By subjecting the sample to various conditions and meticulously observing the resultant outcomes, researchers can discern the influence of different factors on the test's accuracy and reliability. This process contributes to a comprehensive understanding of the test's robustness and performance, aiding in the development and optimization of diagnostic methodologies and enhancing the overall quality of laboratory analyses in the field of clinical diagnostics [7]

The intermediate fidelity outcomes were acceptable across the three levels—low, medium, and high, with coefficients of variation (CV) of 5.38%, 4.16%, and 5.81% respectively. The reproducibility CV for each tier is satisfactory, remaining below the established limits set by both the SFBC (quality control system)

The results have been graphically depicted through Levey-Jennings plots (Fig. 2, Fig. 3, and Fig. 4) to enhance the clarity of the findings.

Table 1 Reproducibility results of blood assay by level with comparison to FSBC and RICOS data

Level of IQC	Number of values	Mean (g/l)	Standard Deviation	Coefficient of Variation CV (%)	CV SFBC 1999 (%)
Low	30	0.04 UI/ml	0.002 UI/ml	5.38 %	20 %
Medium	30	3.89 UI/ml	0.162 UI/ml	4.16 %	7.00 %
High	30	22.24 I/ml	1.292 UI/ml	5.81 %	6.02 %

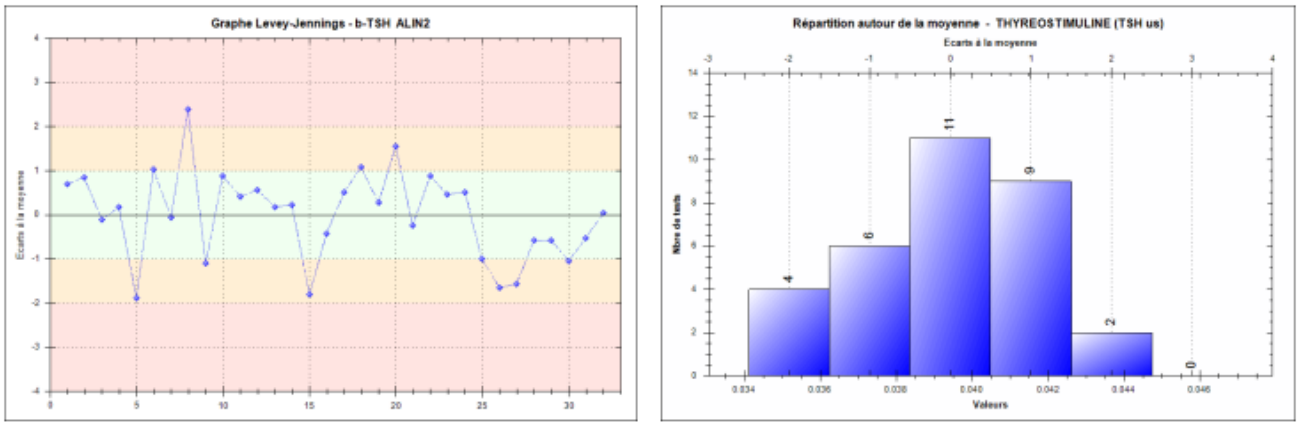

Figure 2 Low Level of Reproducibility: Levey Jennings graph and the distribution around the mean – TSH

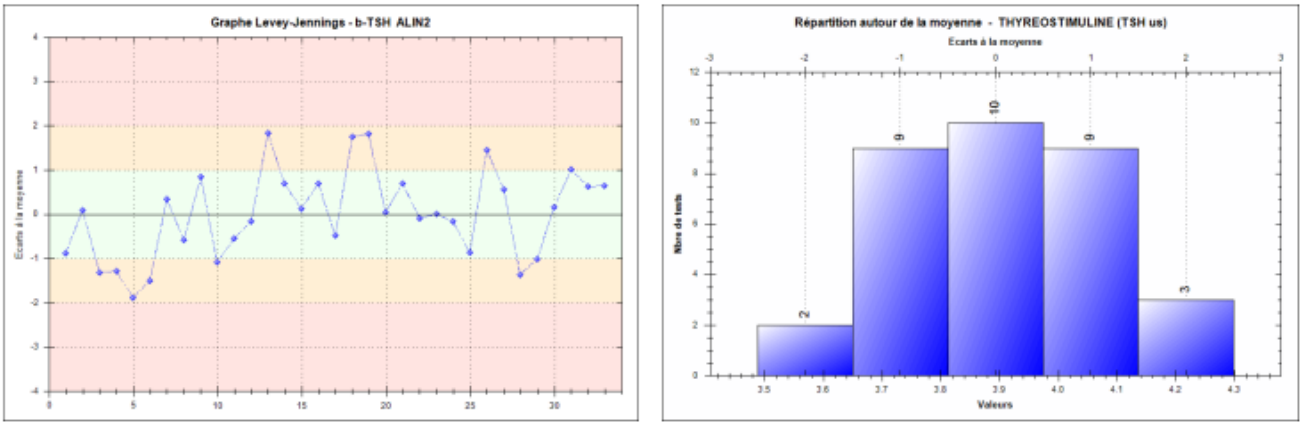

Figure 3 Medium Level of Reproducibility: Levey Jennings graph and the distribution around the mean – TSH

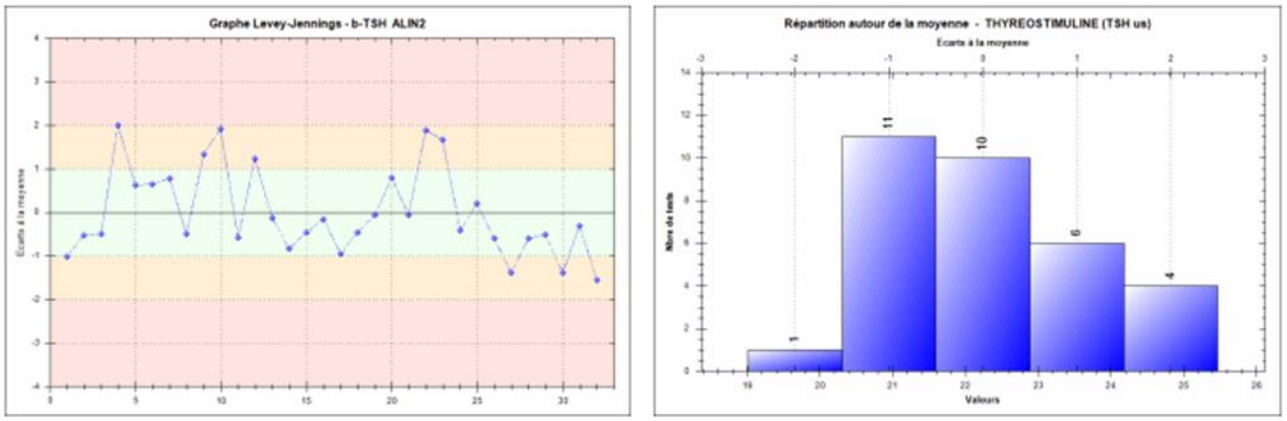

Figure 4 High Level of Reproducibility: Levey Jennings graph and the distribution around the mean – TSH

3.2. Repeatability Results

Repeatability is assessed through the repeated assay of the same samples by the same operator under uniform conditions, encompassing all aspects of the measurements such as reagent, calibration, instrument, and operator and in the briefest time frame possible.

The repeatability test enables the initial performance to be determined and the correct operation of the system (instrument/reagent) to be verified for the analyte concerned (8).

Once more, variability is measured using CV values.

As indicated in Table 2, the results obtained for the various TSH assay verification criteria demonstrate satisfactory repetability for all three levels :low, medium, and high, with coefficients of variation (CV) of CV1 = 2.17%, CV2 = 1.54%, CV3 = 1.98% respectively.

Table 2 Repeatability results for TSH on the Alinity i® automated system by level with comparison to SFBC

Level of IQC	Number of values	Mean (UI/ml)	Standard Deviation (UI/ml)	Coefficient of Variation CV (%)	CV SFBC 1999 (%)
Low	30	0.04	0,001	2,17%	15%
Medium	30	4.06	0,062	1.54%	5,25%
High	30	20.68	0.392	1,90%	3.75%

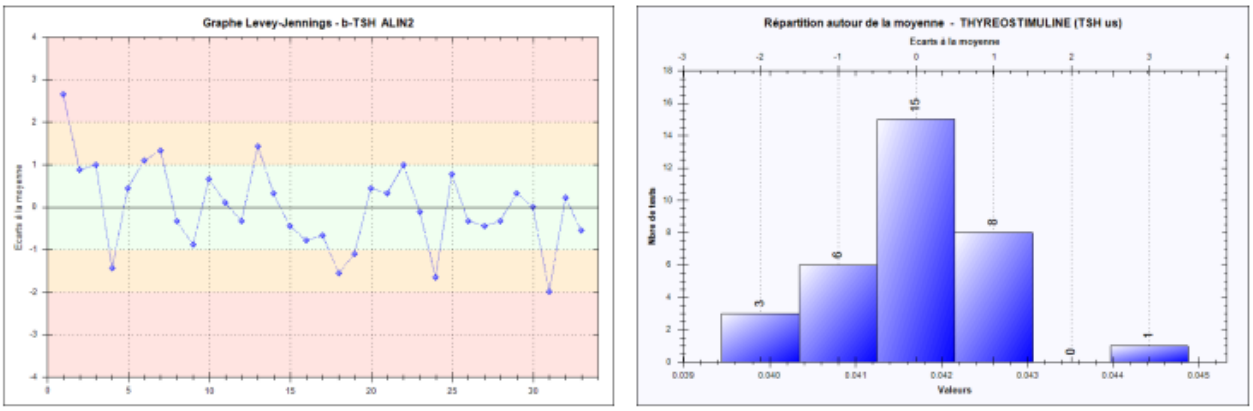

Figure 5 Low Level of Repeatability: Levey Jennings graph and the distribution around the mean – TSH

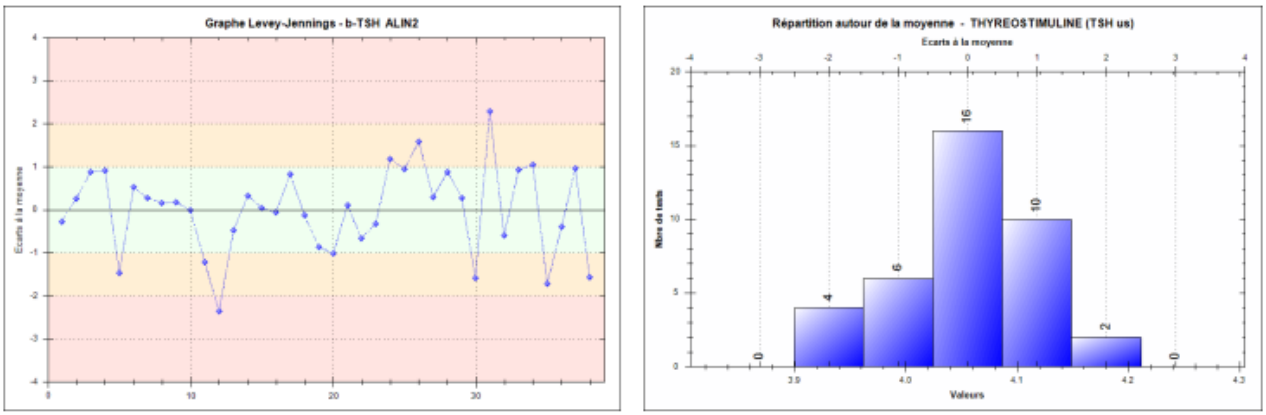

Figure 6 Medium Level of Repeatability: Levey Jennings graph and the distribution around the mean – TSH

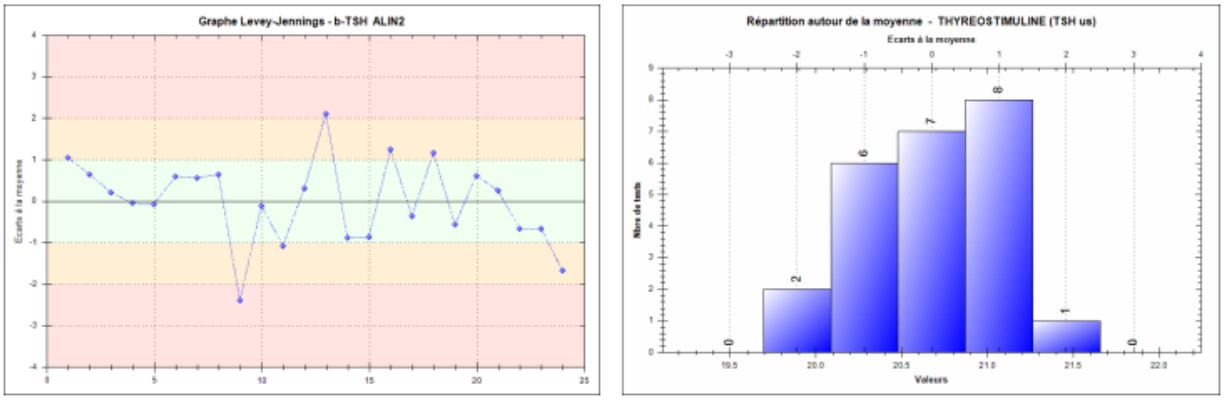

Figure 7 High Level of repeatability: Levey Jennings graph and the distribution around the mean – TSH

4. Discussion

Human Thyroid Stimulating Hormone (TSH) or thyrotropin is a glycoprotein with a molecular weight of approximately 28,000 daltons, synthesized by the basophilic cells (thyrotropes) of the anterior pituitary. TSH is composed of two non-covalently linked subunits designated alpha and beta. Although the alpha subunit of TSH is common to the luteinizing

hormone (LH), follicle stimulating hormone (FSH) and human chorionic gonadotropin (hCG), the beta subunits of these glycoproteins are hormone specific and confer biological as well as immunological specificity. Both alpha and beta subunits are required for biological activity.

TSH stimulates the production and secretion of the metabolically active thyroid hormones, thyroxine (T4) and triiodothyronine (T3), by interacting with a specific receptor on the thyroid cell surface. T3 and T4 are responsible for regulating diverse biochemical processes throughout the body which are essential for normal development and metabolic and neural activity.

The synthesis and secretion of TSH is stimulated by thyrotropin releasing hormone (TRH), the hypothalamic tripeptide, in response to low levels of circulating thyroid hormones. Elevated levels of T3 and T4 suppress the production of TSH via a classic negative feedback mechanism.

Other evidence also indicates that somatostatin and dopamine exert inhibitory control over TSH release, suggesting that the hypothalamus may provide both inhibitory and stimulatory influence on pituitary TSH production. Failure at any level of regulation of the hypothalamic-pituitary-thyroid axis will result in either underproduction (hypothyroidism) or overproduction (hyperthyroidism) of T4 and/or T3.

Sensitive TSH assays now available, with increased ability to clearly distinguish between euthyroid and hyperthyroid populations, are changing thyroid function testing. Analytical sensitivity, as a means of assessing low concentration accuracy, is being replaced by functional sensitivity.

The American Thyroid Association has formally recommended the use of functional sensitivity as the means to quantify the sensitivity of TSH assays, although analytical sensitivity is still widely used. Third generation TSH assays exhibit 20% interassay CVs at < 0.02 μIU/mL and are useful in the discrimination of patients with true hyperthyroidism from those with TSH suppression seen in subclinical hyperthyroidism and some non-thyroidal illnesses.

Other thyroid tests (Free T4 estimate, Total T4, T-Uptake, and Total T3) combined with the ability to accurately measure low levels of TSH, improve the efficiency of thyroid diagnosis.

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 TSH 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 (8).

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. (9)

The reproducibility test is employed to assess the consistency of assay results when different variables are introduced. Our study results affirmed the reliability of the TSH assay for reproducibility assessment. The three levels—low, medium, and high—yielded satisfactory outcomes. For each level, 30 values were analyzed, revealing means of m1 = 0.04 IU/ml, m2 = 3.89 IU/ml, and m3 = 22.24 IU/ml, along with coefficients of variation (CV) of CV1 = 5.38%, CV2 = 4.16%, and CV3 = 5.81%.

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 .(9)

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 three levels (low, medium, and high), 30 values were scrutinized for each level, revealing remarkably low coefficients of variation (CV): CV1 = 2.17%, CV2 = 1.54%, and CV3 = 1.90%. 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. 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 (10) ,(11).

5. Conclusion

For the last few decades, there have been great advances in various methods that are used for detection of TSH elevation (primary hypothyroidism) and low TSH values (hyperthyroidism). The sensitivity improvements in TSH measurements have revolutionized the strategies for investigating thyroid function and have firmly established TSH as the first-line thyroid function test for most clinical situations.

The analytical performance of the alinity ci automated system was satisfactory for a reliable determination of TSH. Verification of methods of dosage in the medical laboratory is crucial to ensure the accuracy, precision, and reliability of laboratory test results. Verification involves confirming that the test method employed is appropriate for the intended use, produces results that are consistent with the claimed performance characteristics, and meets the laboratory's quality control and quality assurance requirements.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Vassault A, Hulin A, Chapuzet E, Arnaud J, Giroud C, membres du sous-groupe 2 analytique de la SFBC. [Verification/validation of the performances of analytical method]. Ann Biol Clin (Paris). déc 2010;68 Spec No 1:247-94.

[2] Pum J. A practical guide to validation and verification of analytical methods in the clinical laboratory. Adv Clin Chem. 2019; 90:215-81.

[3] G. Vassart, J.E. Dumont, The thyrotropin receptor and the regulation of thyrocyte function and growth, Endocr. Rev. 13 (1992) 596e611.

[4] M. Eisenberg, M. Samuels, J.J. DiStefano, Extensions, validation, and clinical applications of a feedback control system simulator of the hypothalamopituitary-thyroid axis, Thyroid 18 (2008) 1071e1085.

[5] S. La Franchi, Graves' disease, congenital hypothyroidism, and maternal thyroid disease during pregnancy, Growth Horm. IGF Res. 16 (2006) S20eS24.

[6] A.C. Bianco, Metabolic effects of thyroid hormones e beyond traditional prospects, Thyroid 18 (2008) 99e100

[7] R. R. Beer et al., « Validation and verification of examination procedures in medical laboratories: opinion of the EFLM Working Group Accreditation and ISO/CEN standards (WG-A/ISO) on dealing with ISO 15189:2012 demands for method verification and validation », Clinical Chemistry and Laboratory Medicine (CCLM), vol. 58, no 3, p. 361-367, mars 2020, doi: 10.1515/cclm-2019-1053.

[8] ISO [Internet]. 2021 [cited 2024 Jan 27]. ISO 15189:2022. Available from: https://www.iso.org/fr/standard/76677.html

[9] Douzi, N., Himri, A., Grari, O., Beyyoudh, S., Mokhtari, S., El Moujtahide, D., ... & Choukri, M. (2024). Verification of the analytical performance of the serum CA 125 assay on the Abbott Alinity ci®: Experience of the biochemistry laboratory of the Mohammed VI university hospital of Oujda. GSC Biological and Pharmaceutical Sciences, 26(2), 230-238.

[10] SEBBAR El-Houcine, SAM Hicham, SIDQI Zaina, et al. Calcium Intakes in the Diet of Eastern Morocco's Population. Journal of Pharmacy and Nutrition Sciences, 2018, vol. 8, no 3, p. 91-96. DOI: https://doi.org/10.6000/1927-5951.2018.08.03.2.

[11] SEBBAR, El-Houcine, SAALAOUI, Ennouamane, CHOUKRI, Mohammed. Evaluation of dietary vitamin D intake in a population in eastern Morocco. Revue Pratiques en Nutrition, 2018, Vol 14 - N 55, p. 44-45 Doi : 10.1016/j.pranut.2018.05.012.

[12] Kumar, K., Mishra, S. K., Dwivedi, P., & Sumana, G. (2019). Recent progress in the sensing techniques for the detection of human thyroid stimulating hormone. TrAC Trends in Analytical Chemistry, 118, 666-676.