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Verification of analytical performance of alpha-fetoprotein assay on the Abbott Alinity ci®: Experience of the central laboratory of Mohammed VI University Hospital of Oujda

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Abstract

The aim of our study was the verification of the analytical performance of alpha-fetoprotein determination on the Abbott CI analyzer utilizing the immuno-chemiluminescence method. The verification process was conducted in the biochemistry laboratory of Mohammed VI University Hospital of Oujda. The working methodology adapted is based on the recommendations of the protocol of the French accreditation committee (COFRAC) accreditation technical guide (GTA) 04, by the evaluation of reproducibility and repeatability. The results obtained by this evaluation were overall satisfactory and have meet the recommended criteria set by supplier and the French society of clinical biology. This study shows that the biochemistry laboratory of Mohammed VI University Hospital of Oujda can deliver an accurate and precise results which can be used for clinical diagnosis and decision making.

Keywords: Alpha-Fetoprotein assay; Analytical performance; Repeatability; Reproducibility; Alinity CI analyzer; Immuno-chemiluminescence

1. Introduction

Alpha-fetoprotein (AFP), an important marker, is an acid glycoprotein with a molecular weight of 69,000, normally found in the fetal liver and yolk sac and gradually disappears after birth. However, it can be detected in several malignant diseases, notably non seminomatous testicular cancer and hepatocellular carcinoma(1). It cas also be used in prenatal screenings for fetal abnormalities. Which make him a crucial biomarker for clinical diagnostics. Its multifaceted role in both oncology and obstetrics underscores the critical need for precise and reliable measurement techniques.

Amidst advancements in laboratory methodologies, the application of chemiluminescent technology in AFP assays has emerged as a promising avenue, offering heightened sensitivity and accuracy in detecting AFP levels.

Our study delves into the crucial process of method verification of the AFP assay using immune-chemiluminescence technology used by Abbott analyzer Alinity Ci. This method involves assessing the analytical performance, measuring them through standardized operational procedures, and then comparing them against criteria set by recognized societies (RICOS, FSCB). This comprehensive approach provides the laboratory with essential insights into its analytical methods, their capabilities, and limitations. Ensuring that these measures meet the standards for delivering reliable analytical outcomes and clinically meaningful interpretations.

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1.1. Interest of alpha-fetoprotein determination

Alpha-fetoprotein is a glycoprotein composed of an α-globulin, encompassing around 600 amino acids, along with a carbohydrate component. Typically found as a monomer (in a single polypeptide chain), AFP weighs approximately 70 kDa. It serves as the primary serum binding protein in the fetus, playing a major role in transporting various substances like hormones, fatty acids, bilirubin, and minerals. During the initial stages of fetal development, AFP is synthesized by the yolk sac—a membranous sac connected to the embryo that functions as the primary circulatory system before the establishment of the formal internal circulation(2).

Alpha-fetoprotein was first described in 1964 by Tatarinov as a human protein associated with tumors(3). It can be secreted by certain germ cell tumors (GCTs) such as non seminomatous testicular cancers, along with primary liver cancers like hepatoblastoma and hepatocellular carcinoma (HCC), and then released into the bloodstream (2,4). As a result, measuring serum AFP has been a routine diagnostic procedure for these tumors since the 1970s. Its significance lies in its role for diagnosis, evaluating treatment response, detecting recurrence, and predicting prognosis. Also, the AFP determination has proven its utility in prenatal screening, it can be measured in the early detection of fetal open neural tube defects (NTD)(2,5).

1.2. Principle of AFP assay method

This assay employs a two-step immunoassay technique to quantitatively measure AFP levels in human serum, plasma, and amniotic fluid, utilizing chemiluminescent microparticle immunoassay (CMIA) technology.

Initially, sample and anti-AFP coated with paramagnetic microparticles are mixed and incubated. The AFP within the sample adheres to the anti-AFP-coated microparticles. Subsequently, the mixture undergoes a washing process. After that, an acridinium-labeled conjugate of anti-AFP is introduced, creating a reaction mixture that is again incubated. Post a wash cycle, the addition of Pre-Trigger and Trigger, then solutions follows.

The resultant chemiluminescent reaction is gauged in relative light units (RLUs). The system optics detect RLUs, showing a direct correlation between the AFP quantity in the sample and the RLUs observed.

2. Materials 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 alpha-fetoprotein values across the full measurement spectrum. These collected samples were categorized into three groups representing low, medium, and high AFP levels. To gauge repeatability, each serum sample underwent 30 individual assay runs.

The AFP 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. Intermediate fidelity results

The outcomes of the intermediate fidelity examination yielded satisfactory results for all levels low, medium, and high levels, yielding coefficients of variation (CV1, CV2 and CV3) of 5.14%, 3.44% and 3.33% respectively (Table 1).

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

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

Level of IQC	Numbers of value	Mean (ng/ml)	Standard deviation	Coefficient of variation CV (%)	Reference CV: FSBC 1999	References CV: RICOS (%)
Low	30	4.36	0.224	5.14%	15.0%	12.2%
Medium	30	73.83	2.541	3.44%	8.0%	12.2%
High	30	208.28	6.939	3.33	8.0%	12.2%

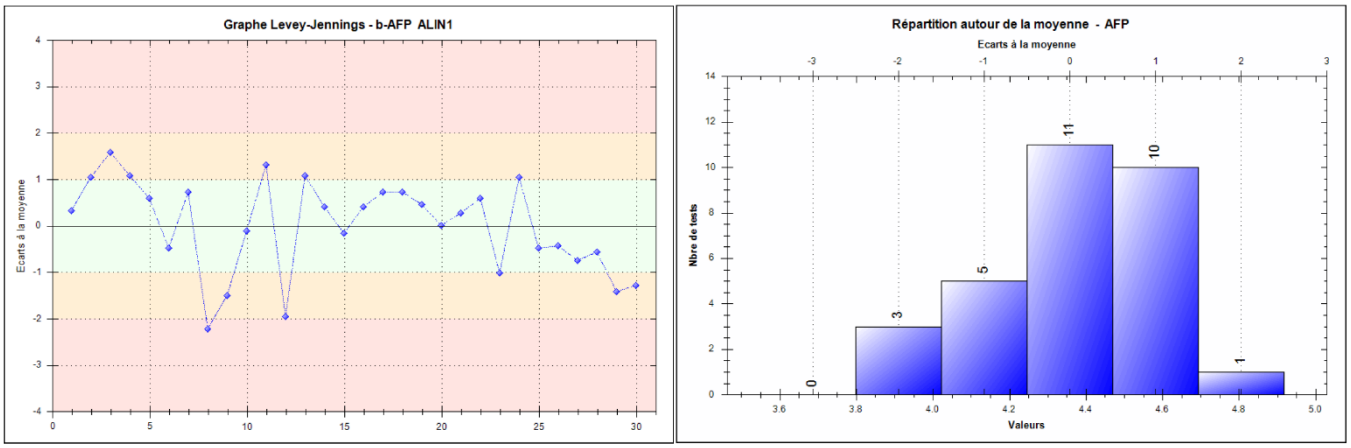

Figure 1 Low Level of reproducibility : Levey Jennings graph and the distribution around the mean

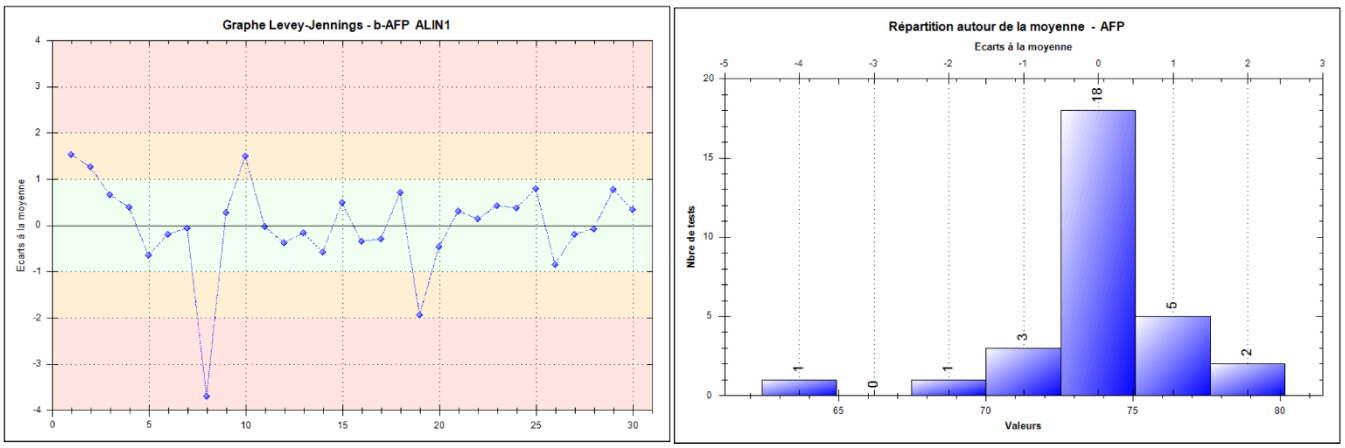

Figure 2 Medium Level of reproducibility : Levey Jennings graph and the distribution around the mean

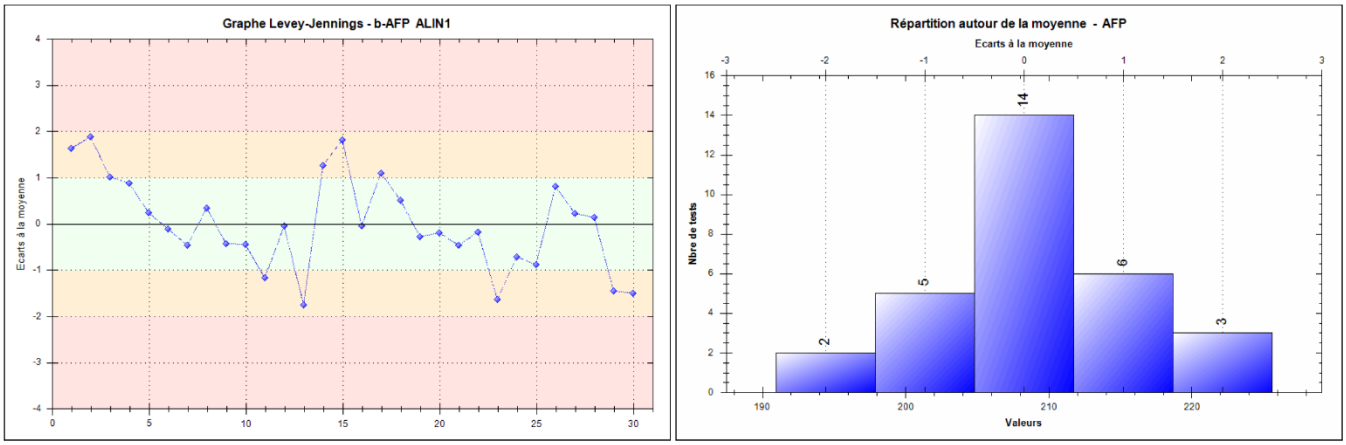

Figure 3 High Level of Reproducibility : Levey Jennings graph and the distribution around the mean

3.2. Repeatability results

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 2).

These findings are visually expounded upon through Levey Jennings plots, illustrating the results in a more comprehensive manner (Fig. 4, Fig. 5, and Fig 6).

Table 2 Repeatability results of blood assay by level with comparison to FSBC and RICOS data

Level of IQC	Number of value	Mean (ng/ml)	Standard deviation	Coefficient of variation CV (%)	Reference CV: FSBC 1999	References CV: RICOS (%)
Low	30	5.38	0.075	1.39 %	11.3%	12.2%
Medium	30	65.08	1.431	2.20%	6.0%	12.2%
High	30	168.22	3.896	2.32 %	6.0%	12.2%

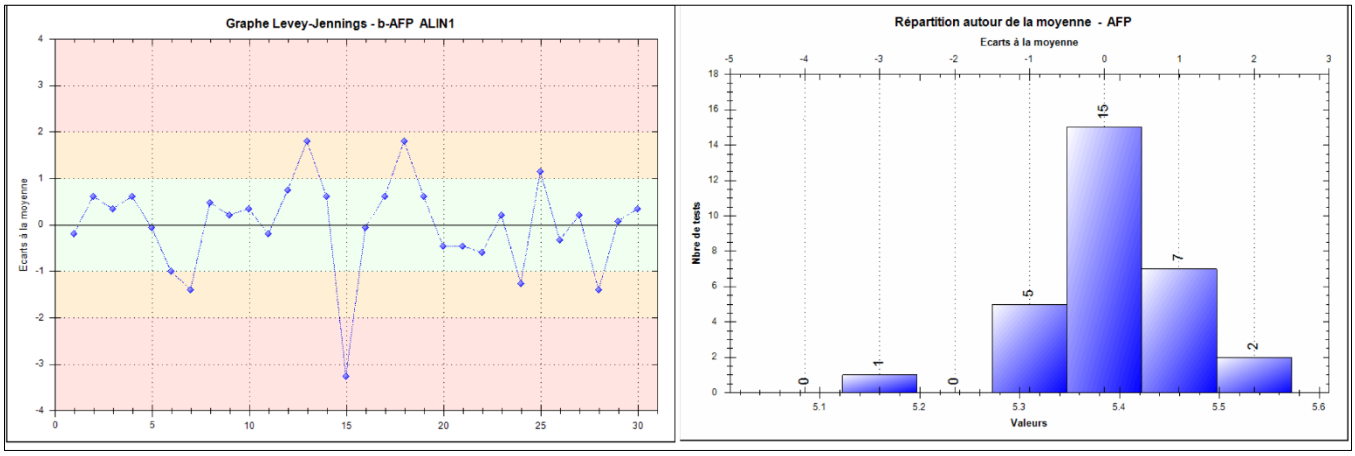

Figure 4 Low Level of Repeatability: Levey Jennings graph and the distribution around the mean

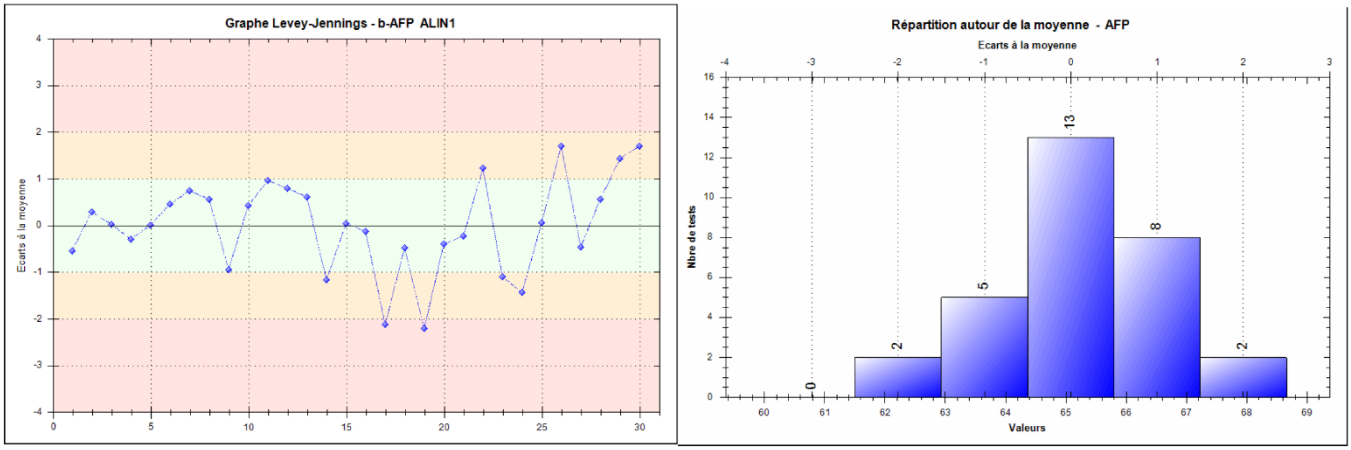

Figure 5 Medium Level of Repeatability: Levey Jennings graph and the distribution around the mean

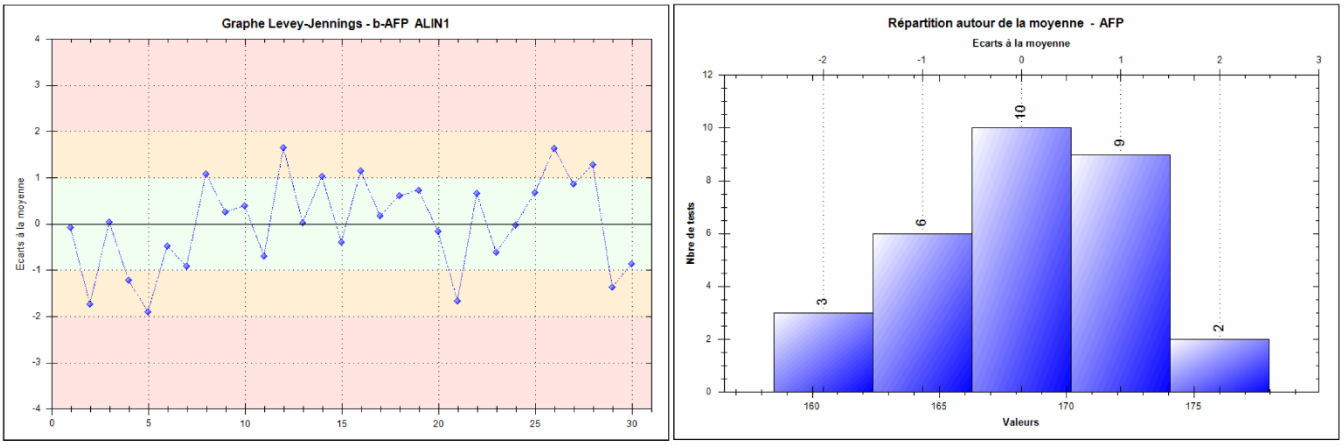

Figure 6 High Level of Repeatability: Levey Jennings graph and the distribution around the mean

4. Discussion

AFP, an oncofetal glycoprotein, is one of the most commonly tested tumor biomarkers. While a slight increase in serum AFP levels may occur in individuals with liver cirrhosis, hepatitis, or during pregnancy, notably elevated levels are observed in patients with conditions such as HCC and non-seminomatous testicular cancer(6). These increased levels prove instrumental in diagnosing, categorizing, staging, and monitoring these diseases. Thus, an accurate and precise results must be delivered to practitioners.

The verification/validation of methods within a medical laboratory is a crucial process that ensures precise and reliable measurements. It serves as 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(7,8). The AFP assay, utilizing the immunochemiluminescent method, is presently a validated method, thus necessitating verification rather than validation. In this study, we conducted a verification of the analytical performance of the alpha-fetoprotein assay on the AbbottAlinity CI analyzer by using the COFRAC guide SH-GTA-04(9).

Repeatability and intermediate fidelity are statistical methods utilized in process control to measure precision and variability within our automated systems. The intermediate fidelity test, also known as intra-laboratory reproducibility, involves examining a single sample under various conditions by altering at least one factor: such as the operator, timing, reagent kits, or calibrations. This process helps establish acceptance criteria in line with prior data, considering biological variations. This is especially valuable in decision support systems(10,11).

The results of intermediate fidelity evaluation suggests that the AFP assay, employing the immune-chemiluminescent method, has shown consistency and agreement in measurements across different conditions. Which suggest that the technique is robust and reliable, and it can be trusted for clinical diagnosis.

As for, the repeatability test, it involves analyzing a single sample under precise conditions, including the same operator, reagent kits, instrument, and calibration, all completed within the shortest feasible timeframe(12). This process aims to characterize the optimal performance of the system (instrument/reagent) for the specific analyte, ensuring the verification of its proper functioning under these controlled conditions. For a given analyzer, this calculation must be performed for each analyte/matrix to be measured and at several concentration levels. The levels are chosen according to the medical decision areas(11,13).

The results of the repeatability evaluation have demonstrated that the immune-chemiluminescent AFP assay utilized in our laboratory exhibits high precision. This is substantiated by consistently low coefficients of variation for repeatability, showcasing minimal variability in repeated measurements conducted under identical conditions. This results not only emphasizes the reliability this method but also underscores the stability and robustness.

The Coefficients of variation and Standard deviations obtained from the analysis of repeatability and the intermediate fidelity were highly acceptable. fulfilling both the supplier's stipulated requirements and the criteria outlined in the SFBC Valtec protocol and RICOS. These findings affirm that the immuno-chemiluminescent method used by the biochemistry laboratory of Mohammed VI University hospital AFP determination on the Abbott Alinity CI analyzer for is not only consistent but also stable in replicating precise measurements across different concentration levels. The alignment of these results with SFBC and RICOS standards substantiates the method's robustness and reliability, signifying its suitability for accurate and dependable measurements in critical clinical diagnostics.

5. Conclusion

In summary, our study thoroughly verifies the analytical performance of the immuno-chemiluminescent AFP assay on the Abbott Alinity CI analyzer. The results obtained from this verification affirm an exceptional precision, consistency and stability, meeting both the supplier requirements and SFBC Valtec protocol standards. This shows that the method used by our laboratory is reliable and can be employed for clinical diagnostics and decision-making.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declares that they have no known competing financials interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Li J, Gao T, Gu S, Zhi J, Yang J, Li G. An electrochemical biosensor for the assay of alpha-fetoprotein-L3 with practical applications. Biosens Bioelectron. 2017 Jan 15, 87:352–7.

[2] Murray MJ, Nicholson JC. α-Fetoprotein. Arch Dis Child Educ Pract Ed. 2011 Aug, 96(4):141–7.

[3] Tatarinov IS. [DETECTION OF EMBRYO-SPECIFIC ALPHA-GLOBULIN IN THE BLOOD SERUM OF A PATIENT WITH PRIMARY LIVER CANCER]. Vopr Med Khim. 1964, 10:90–1.

[4] Prognostic factors in advanced non-seminomatous germ-cell testicular tumours: results of a multicentre study. Report from the Medical Research Council Working Party on Testicular Tumours. Lancet Lond Engl. 1985 Jan 5, 1(8419):8–11.

[5] Poloce F, Boisson-Gaudin C. Marqueurs sériques maternels d'anomalies fœtales (trisomie 21, anomalies chromosomiques, spina bifida,…). Rev Francoph Lab. 2010 Apr 1, 2010(421):59–68.

[6] Murugavel KG, Mathews S, Jayanthi V, Shankar EM, Hari R, Surendran R, et al. Alpha-fetoprotein as a tumor marker in hepatocellular carcinoma: investigations in south Indian subjects with hepatotropic virus and aflatoxin etiologies. Int J Infect Dis. 2008 Nov 1, 12(6):e71–6.

[7] E. Masson, « Guide de bonne exécution des analyses de biologie médicale (GBEA) : son application dans le secteur hospitalier public »,]. Vol 1999 - N° 309.

[8] ISO 15189:2022 », Medical laboratories Requirements for quality and competence.

[9] Cofrac SH GTA 04, Guide technique d'accréditation de vérification (portée A) /validation (portée B) des méthodes de Biologie médicale .

[10] Mokhtari I, Elkhamlichi IE, Kajeiou Z, Dahmani S, Saddari A, Sebbar EH, et al. Verification of the analytical performance of the serum glucose assay on the Abbott Alinity ci®. GSC Biol Pharm Sci. 2023, 24(3):068–74.

[11] Abdel Ghafar MT, El-Masry MI. Verification of quantitative analytical methods in medical laboratories. J Med Biochem. 2021 Jun 5, 40(3):225–36.

[12] Kajeiou Z, Yacoubi L, Mokhtari I, Dahmani S, Sebbar E houcine, Choukri M, et al. Verification of the analytical performance of the serum total cholesterol assay on Abbott Architect ci8200. World J Biol Pharm Health Sci. 2023, 15(2):104–13.

[13] Sbibih Y, Merimi I, Houcine SEBBAR E, Choukri M. Verification of the analytical performance of the serum albumin assay on the Abbott Alinity ci. Mater Today Proc. 2023 Jan 1, 72:3857–62.