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(RESEARCH ARTICLE)

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Verification of the analytical performance of the anti-cyclic citrullinated peptide assay on ALINITY ci ® experience from the biochemistry laboratory of Mohammed VI University Hospital in Oujda

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Abstract

The aim of our work was to evaluate the analytical performance of the anti-cyclic citrullinated peptide 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 two levels (low and high) both for intermediate fidelity, with coefficients of variation (CV) of CV1= 1.9% and CV2=1.8% respectively, and for repeatability, with coefficients of variation of CV1 = 1.7%, CV2= 1.61%. 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: Anti-cyclic citrullinated peptide; 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 manufacturer, 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.

The anti-CCP test is more specific than the commonly used rheumatoid factor RF test (95% versus less than 90%) and has a comparable sensitivity (more than 70%). These antibodies are detectable very early in the disease and are reported to predict the development of erosive rheumatoid Arthritis RA (3).

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In this study, we carried out a method verification protocol for anti-CCP, 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 anti-CCP

Anti-cyclic citrullinated peptide antibodies (anti-CCP) are autoantibodies targeting peptides containing citrulline, an amino acid that results from the deimination of l-arginine. These antibodies play a crucial role in the diagnosis of rheumatoid arthritis (RA), an autoimmune disease that causes chronic inflammation of the joints

Citrullination is a post-translational modification of proteins, where l-arginine is converted to citrulline by the action of peptidylarginine deiminase (PAD) enzymes. The citrullinated peptides thus formed can trigger an immune response, leading to the production of anti-CCP antibodies. Anti-CCP antibodies are particularly specific to rheumatoid arthritis, which distinguishes them from other immunological markers. A positive result strongly suggests the presence of rheumatoid arthritis, while an indeterminate result requires monitoring and additional tests. A negative result does not completely exclude RA, but decreases its likelihood

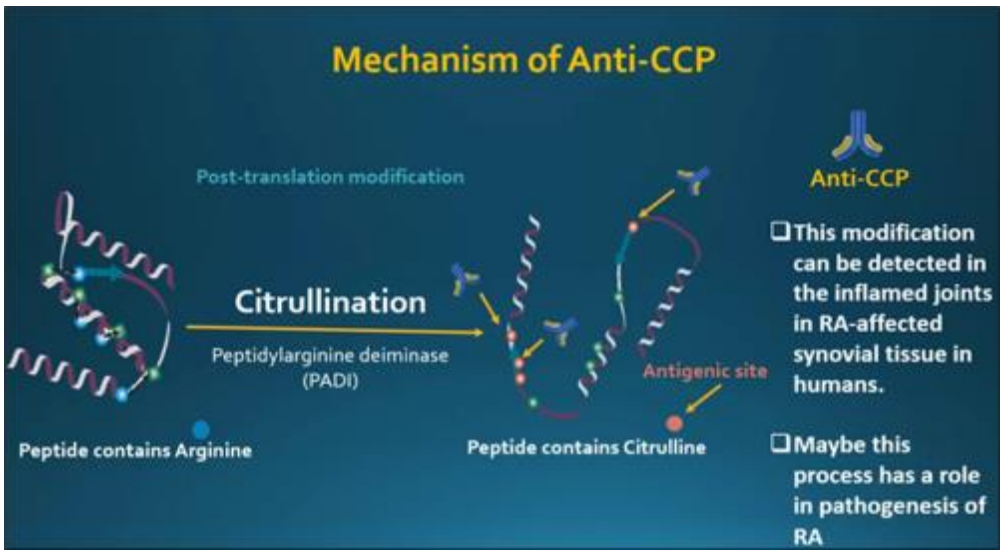

Figure 1 Mechanism of anti-CCP

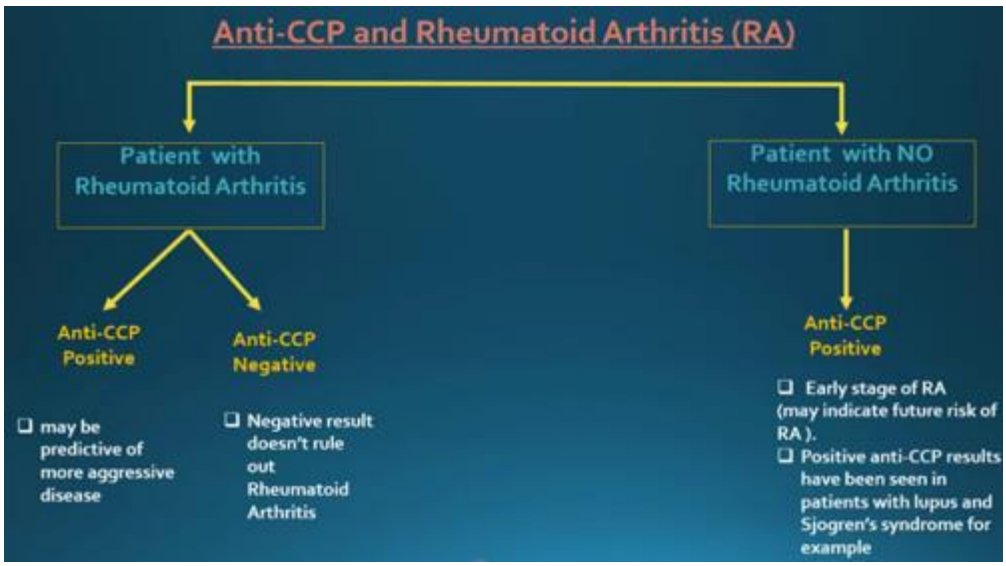

Figure 2 Interpretation of anti-CCP results

1.2. Principle of the assay method

This assay is a two-step immunoassay with an automated sample pretreatment for the semi-quantitative determination of the IgG class of autoantibodies specific to cyclic citrullinated peptide (CCP) in human serum or plasma using chemiluminescent microparticle immunoassay (CMIA) technology. Sample and wash buffer are combined. An aliquot of the prediluted sample, CCP coated paramagnetic microparticles, and sample diluent are combined and incubated. The anti-CCP antibodies present in the sample bind to the CCP coated microparticles. The mixture is washed. Anti-human IgG acridinium-labeled conjugate is added to create a reaction mixture and incubated. Following a wash cycle, Pre-Trigger and Trigger Solutions are added. The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is a direct relationship between the amount of anti-CCP antibody in the sample and the RLUs detected by the system optics.

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 two concentration levels—low 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 anti-CCP values across the full measurement spectrum. These collected samples were categorized into two groups representing low and high anti-CCP levels. To gauge repeatability, each serum sample underwent 30 individual assay runs.

The anti-CCP 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 manufacturer's specifications. , 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. 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 (4)

Table 1 Reproducibility results of blood assay by level with comparison to manufacturer’s specifications

Level of IQC	Number of values	Mean (g/l)	Standard Deviation	Coefficient of Variation CV (%)	CV manufacturer’s specifications (%)
Low	30	0.74 UI/ml	0.058 UI/ml	1.9 %	2.1 %
High	30	25.19 UI/ml	1.845 UI/ml	1.8 %	2.3 %

The intermediate fidelity outcomes were acceptable across the two levels—low, and high, with coefficients of variation (CV) of 1.9% and 1.8% respectively. By comparing these results with the CV retained by manufacturer’s specifications, we note that the results are in conformity and inferior to the tolerated limits.

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

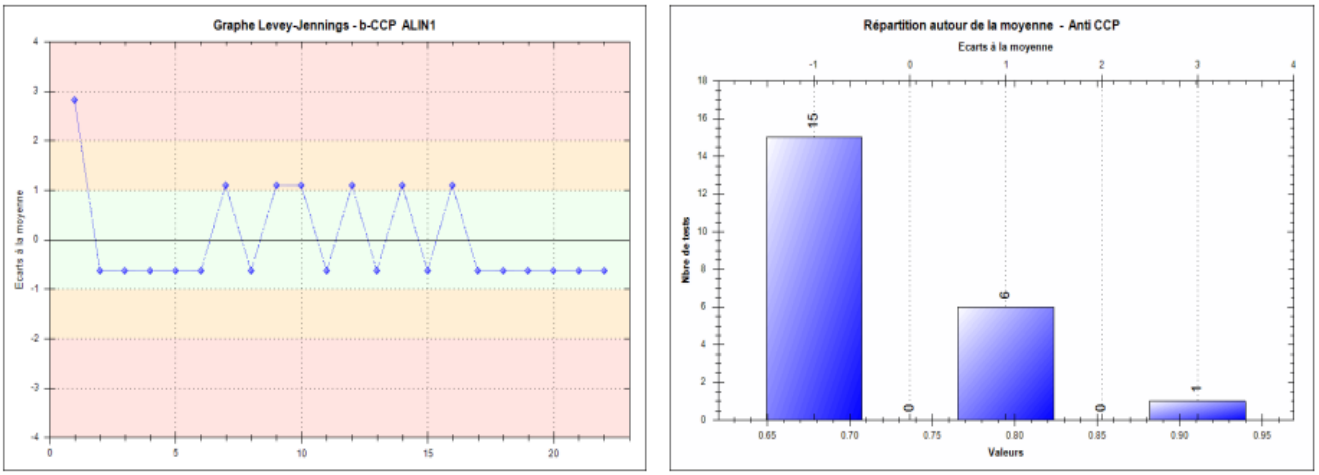

Figure 3 Low Level of Reproducibility: Levey Jennings graph and the distribution around the mean – anti-CCP

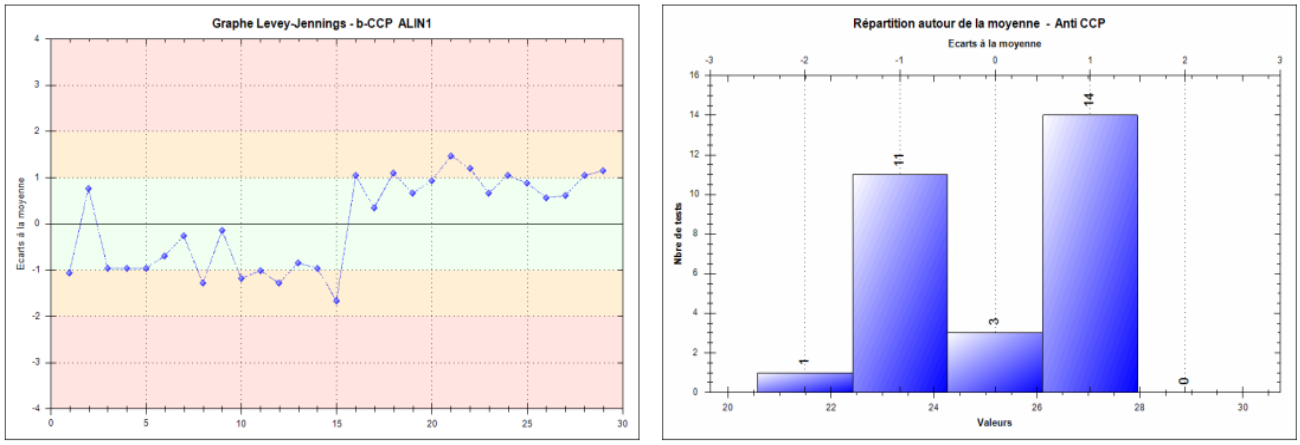

Figure 4 High Level of Reproducibility: Levey Jennings graph and the distribution around the mean – anti-CCP

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 (5). Once more, variability is measured using CV values.

As indicated in Table 2, the results obtained for the various anti-CCP assay verification criteria demonstrate satisfactory repetability for two levels :low,and high, with coefficients of variation (CV) of CV1 = 1.7%, CV2= 1.61% respectively.

Table 2 Repeatability results for anti-CCP on the Alinity i® automated system by level with comparison to manufacturer's specifications

Level of IQC	Number of values	Mean (UI/ml)	Standard Deviation (UI/ml)	Coefficient of Variation CV (%)	CV SFBC 1999 (%)
Low	30	0.68	0,054	1.7%	1.9%
High	30	23	0.369	1,61%	1.9%

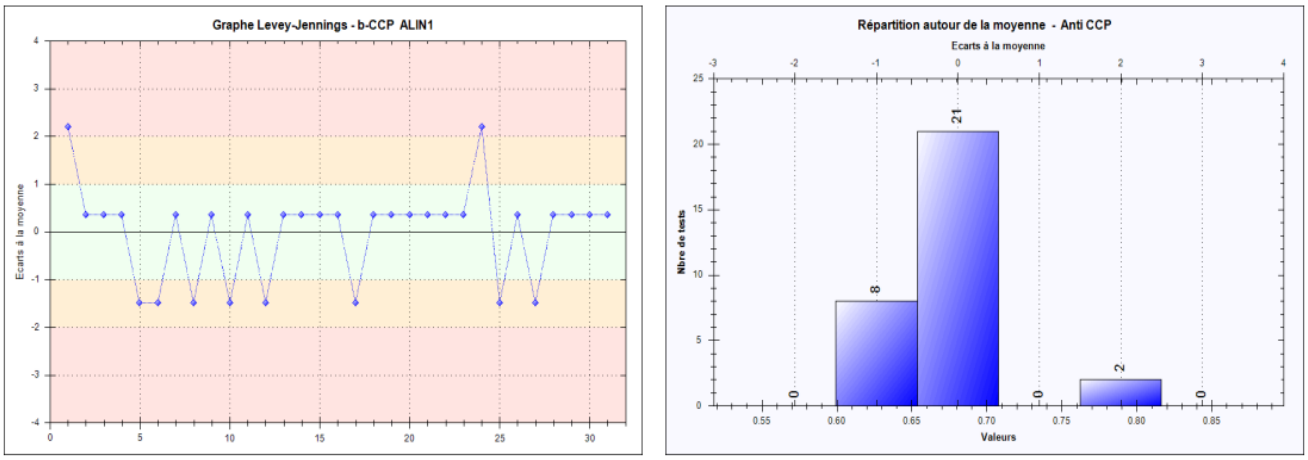

Figure 5 Low Level of Repeatability: Levey Jennings graph and the distribution around the mean – anti-CCP

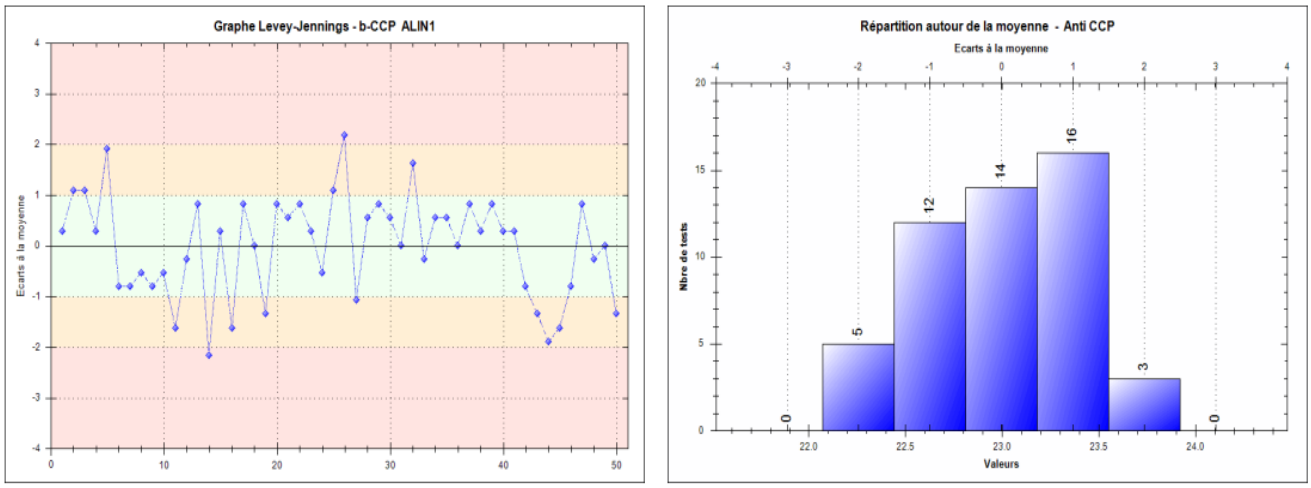

Figure 6 High Level of repeatability: Levey Jennings graph and the distribution around the mean – anti-CCP

4. Discussion

Rheumatoid Arthritis (RA) is a common, systemic autoimmune disease affecting 0.5-1% of the population. It is characterized by chronic inflammation of the synovium, which commonly leads to progressive joint destruction and in most cases, to disability and reduction of quality of life.Evidence gained over the last few years suggests that aggressive therapy given early in the disease has the greatest therapeutic potential(6).

The serum of RA patients contains a variety of antibodies directed against self-antigens. The most widely known of these autoantibodies is the rheumatoid factor (RF) antibody directed against the constant domain of IgG molecules. The presence of RF is one of the American College of Rheumatology's (ACR) criteria for the classification of RA(7).

Although the RF test has good sensitivity for RA, it is not very specific for the disease as it can also be detected in the serum of patients with other rheumatic or inflammatory diseases and even in a substantial percentage of the healthy (elderly) population (8) For several years it has been recognized that antibodies to anti-perinuclear factor (APF) and anti-keratin (AKA) are highly specific for RA. It was subsequently reported that both of these antibodies reacted with native filaggrin and are now referred to as anti-filaggrin antibodies (AFA). More recently it has been shown that all of these antibodies are directed to citrulline-containing epitopes.9

Citrulline is a non-standard amino acid, as it is not incorporated into proteins during protein synthesis. It can, however, be generated via post-translational modification of arginine residues by the enzyme peptidyl arginine deiminase (PAD).

In 1998, Schellekens and colleagues reported that linear peptides containing citrulline (CP) were very specific for RA antibodies (96%) in an ELISA based assay. Subsequent work demonstrated that cyclic variants of these peptides, termed cyclic citrullinated peptides (CCP), were equally specific for RA, but with a higher sensitivity than linear peptides.

To improve the sensitivity of the CCP test further, several dedicated libraries of citrulline-containing peptides were screened with RA sera and a new set of peptides (CCP2) were discovered which gave superior performance compared to the CCP1 test(10) Over the last few years, many independent studies have confirmed the diagnostic performance of the CCP2 test.

In 2007, the European League against Rheumatism (EULAR) published guidelines for the diagnosis of early RA, and the measurement of antibodies to anti-CCP was included as a serology marker.

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 anti-CCP 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 (11).

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 (12).

The reproducibility test is employed to assess the consistency of assay results when different variables are introduced.(15) Our study results affirmed the reliability of the anti-CCP assay for reproducibility assessment. The two levels—low and high—yielded satisfactory outcomes. For each level, 30 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 = 1.9% and CV2 =1.8%

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 (12).

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 = 1.7% and CV2 = 1.61%. 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 (16). 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 (13)(14).

5. Conclusion

It is now demonstrated that a background treatment initiated during the first six months of rheumatoid arthritis can prevent or limit the characteristic joint destruction of this serious pathology. Tools for the early diagnosis of this rheumatism are therefore of major interest. This is the case with the anti-CCP test. Relatively recent in appearance, these

tests are distinguished notably by their high specificity and constitute a good predictive tool for the progression of the disease

The analytical performance of the alinity ci automated system was satisfactory for a reliable determination of anti-CCP. 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.

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