

Verification of the analytical performance of CA 19-9 assay on Abbott Alinity ci®: Experience of the central laboratory Mohammed VI Oujda

Sabah Mokhtari ^{1,2,*}, Amina Himri ^{1,2}, Loubna Yacoubi ^{1,2}, Soufiane Beyyoudh ^{1,2}, Dounia El Moujtahid ^{1,2}, El houcine Sebbar ^{1,2} and Mohammed Choukri ^{1,2}

¹ Central Laboratory, Mohammed VI University Hospital, Oujda, Morocco.

² Mohammed First University, Faculty of Medicine and Pharmacy of Oujda, Morocco.

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Abstract

Introduction: CA19-9 is a marker used in the diagnosis and monitoring of several cancer diseases. The aim of this study is to evaluate the CA19-9 assay on the Abbott ALinity Ci automated system. This evaluation is part of an overall approach to verifying the methods used in the CHU's central laboratory, with a view to compiling an accreditation file in line with the requirements of standard NF EN ISO 15189.

Materials and methods: The aim of our study was to evaluate the scope A criteria detailed in the guide to verification/validation of methods in Medical Biology, in accordance with the recommendations of standards NF EN ISO/CEI 17025, NF EN ISO 15189 and NF EN ISO 22870. Verification was carried out on the CA19-9 assay on Alinity® Ci, which uses the immunoassay technique. Criteria (repeatability, reproducibility) were verified.

Results: The results obtained show good repeatability for the 3 levels with respectively CV1=2,61% , CV2=2,78% , and CV3 =3,26% % and intra-laboratory reproducibility with CV1=3,98% , CV2= 3,16% and CV3 = 3,61%.

Discussion and Conclusion: The central laboratory of our University Hospital is committed to a quality policy which includes a control approach for the various analytical systems used. The results obtained for the various CA19-9 assay verification criteria on our Alinity® Ci system, compared with data from the supplier, RICOS and learned societies, are satisfactory and verify analytical performance.

Keywords: CA 19-9(carbohydrate antigen); Verification; Repeatability; Reproducibility; Alinity ci®

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).

Tumor markers are useful for screening, diagnosis, monitoring and evaluating the treatment and prognosis of cancer patients. Among the many tumor markers, carbohydrate antigen 19-9 (CA 19-9) has relatively high sensitivity and specificity for pancreatic and biliary tract tumors (3). CA 19-9 is of diagnostic value, either when the patient presents

* Corresponding author: Sabah Mokhtari

symptoms (weight loss, abdominal pain and jaundice), or when imaging studies indicate a tumor. However, in asymptomatic patients, cancer screening cannot be entrusted to this marker alone (3).

In this study, we carried out a method verification protocol for a biomarker, CA19-9, 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. Interest of CA 19-9 determination

CA 19-9 carbohydrate antigen, also known as sialyl Lewis a (sLe a), was first identified by Koprowski and colleagues (in Philadelphia in 1979(4), and Lewis antigen is essential for its biosynthesis, Patients who are Lewis negative (5-10% of the population) do not secrete this marker (4). CA19-9 is the reference marker for pancreatic adenocarcinomas, and has been applied to predict the malignancy of intracanal papillary mucinous neoplasms (IPMN) and pancreatic neuroendocrine neoplasms (pNEN), two common types of pancreatic tumors with a low degree of malignancy (4). In addition, CA19-9 is used as a biomarker in various types of cancer, including cancers of the digestive tract, stomach cancer, colorectal cancer, biliary cancer, lung cancer and thyroid cancer (3).

This antigen is a glycoprotein (high molecular weight) which is detected on the surface of certain cancer cells and in the blood (by its release). However, this test alone cannot be used to detect pancreatic or other cancers, since it may be present in small quantities in the pancreas, liver, gallbladder and lungs of healthy adults, and can often be elevated in benign pancreatobiliary diseases such as cholangitis, and pancreatitis explained by inflammation and also by blocked excretion of CA 19-9 in these diseases, elevated CA 19-9 levels can be found in other benign conditions such as lung disease (bronchiectasis ,idiopathic pulmonary fibrosis) and thyroid disease, Hepatitis, diabetes mellitus and chronic glomerulonephritis due to metabolic dysfunction (5-6), and gynecological diseases (ovarian cyst, endometriosis) (5). In contrast, in patients with an elevated CA 19-9 level, associated with an abdominal computed tomography (CT) scan suspicious of malignancy, it is usually sufficient to detect these cancers. (5-7-8).

1.2. Principle of the Alinity ci CA 19-9 method

The Alinity ci CA 19-9XR CMIA assay uses an antigen-antibody system based on the 1116-NS-19-9 antibody, and is a chemiluminescence microparticle immunoassay (CMIA) used for the quantitative determination of 1116-NS-19-9 reactive determinants in human serum or plasma on the Alinity ci analyzer, this assay is performed in two steps: The sample and 1116-NS-19-9 antibody-coated paramagnetic microparticles are brought together and incubated. The 1116-NS-19-9 reactive determinants present in the sample bind to the 1116-NS-19-9 antibody-coated microparticles, after washing, the acridinium-labeled 1116-NS-19-9 conjugate is added to form a reaction mixture, then incubated. After another wash cycle, the pre-activation and activation solutions are added. The resulting chemiluminescent reaction is measured in relative light units (RLU) by the optical system (direct relationship between the amount of 1116-NS-19-9 reactive determinants present in the sample and RLU) (9).

2. Materials and methods

Verification procedure: The biochemistry laboratory of the university hospital Mohammed VI of Oujda carries out rigorous verification of the analytical performance of CA 19-9 marker assay kits using the "Immunological" module of the Abbott Alinity ci automated system.

The verification process meticulously follows the flexible A-scope, which involves comprehensive verification of all elements to ensure accurate and reliable results. An in-depth performance study was carried out on the Abbott Alinity ci PLC, evaluating key parameters such as repeatability, reproducibility, to ensure consistent, reliable results. These techniques are classified in Scope A for method verification, as indicated in COFRAC guide SH-GTA-04. Standard deviations (SD) and coefficients of variation (CV) were carefully evaluated and analyzed during the performance evaluation process, in accordance with the standards set by the French Society of Clinical Biology (SFBC) to ensure that the performance of the Abbott Alinity ci automated system used in the biochemistry laboratory at the university hospital center Mohammed VI of Oujda was in line with industry references and guidelines. Data were statistically processed using BYG informatics' EVM middleware module, a gateway application between the Alinity c automated system and the ilab results validation software.

3. Results

The results reveal satisfactory repeatability for Abbott Alinity ci® at low, medium and high levels, as shown by coefficients of variation (CV) of 2.61%, 2.78%, 3.26 % respectively, as presented in the following figures Repeatability results for CA 19-9 on Alinity ci automaton.

Table 1 Repeatability results of blood assay of CA 19-9(Alinity ci) by level with comparison to FSBC and RICOS data (with expansion coefficient k = 1.211)

| Level of IQC | Number of values | Mean UI /ml | Standard Deviation | Coefficient of Variation CV (%) | Reference CV: FSBC 1999 (%) | Reference CV: RICOS 2014 (%) |
|--------------|------------------|-------------|--------------------|---------------------------------|-----------------------------|------------------------------|
| Low | 31 | 38.14 | 0.995 | 2.61% | 10.87% | 7.25 % |
| Medium | 32 | 66.60 | 1.848 | 2.78% | 9.03% | 7.23% |
| High | 33 | 147.82 | 4.814 | 3.26 % | 9.01 % | 7.21 |

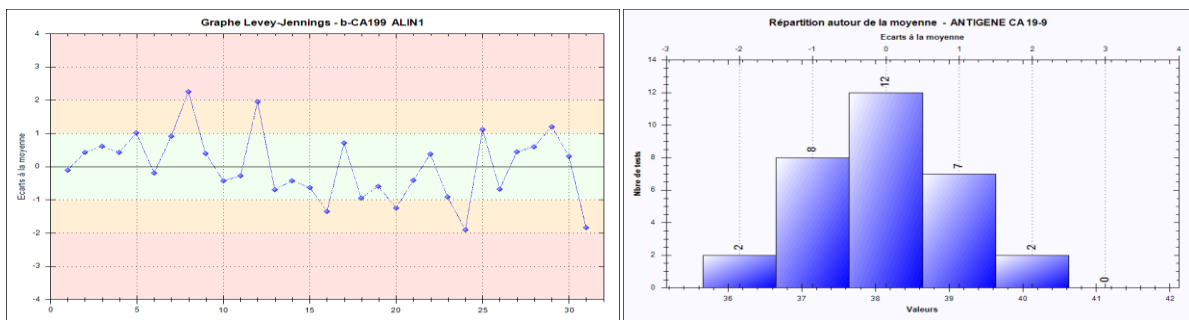


Figure 1 Low Level of Repeatability: Levey Jennings graph and the distribution around the mean-CA19-9

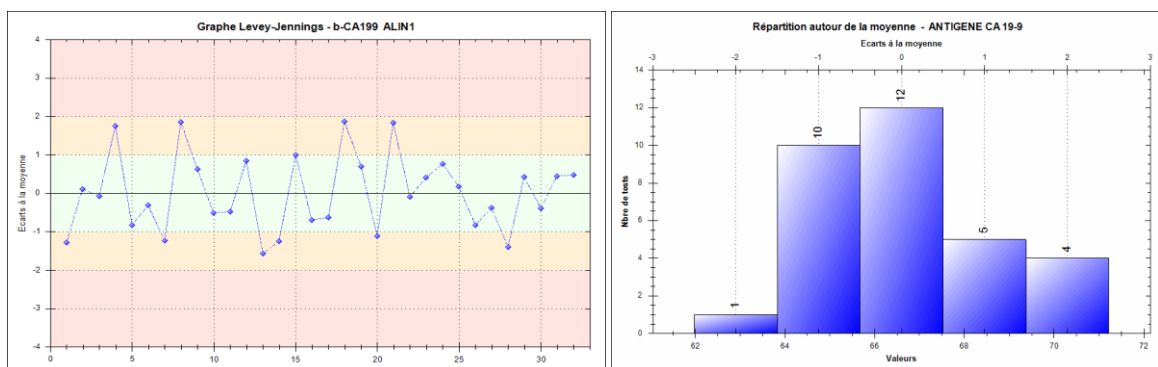


Figure 2 Medium Level of Repeatability: Levey Jennings graph and the distribution around the mean

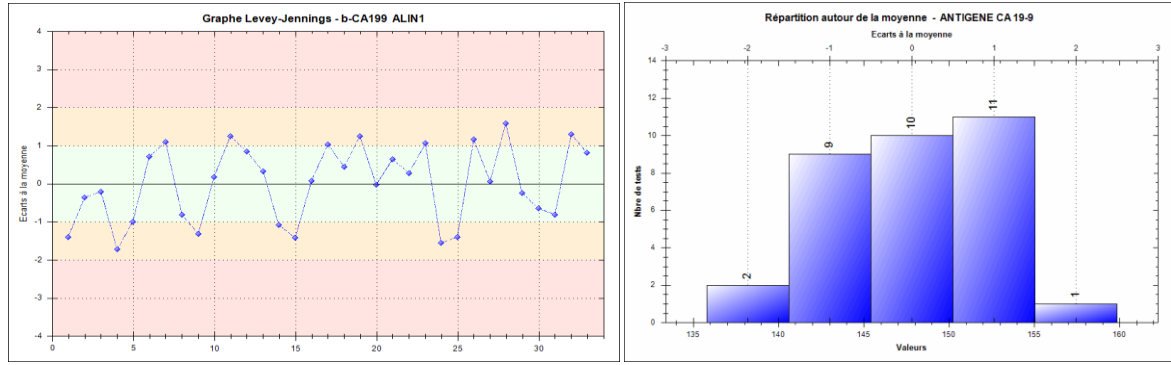


Figure 3 High of Repeatability: Levey Jennings graph and the distribution around the mean

The intra-laboratory reproducibility of Alinity ci® was found to be acceptable for levels 1, 2 and 3, with coefficients of variation (CV) corresponding to 3.98%, 3.16%, 3.61% respectively, as shown in figures 4 to 6.

3.1. Reproducibility results for CA 19-9 on Alinity ci automaton

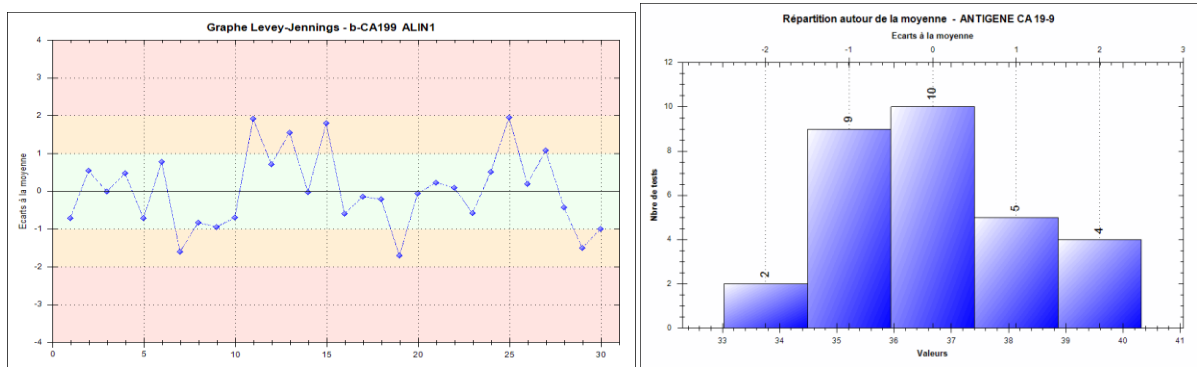


Figure 4 Low Level of Reproducibility: Levey Jennings graph and the distribution around the mean –CA 19-9.

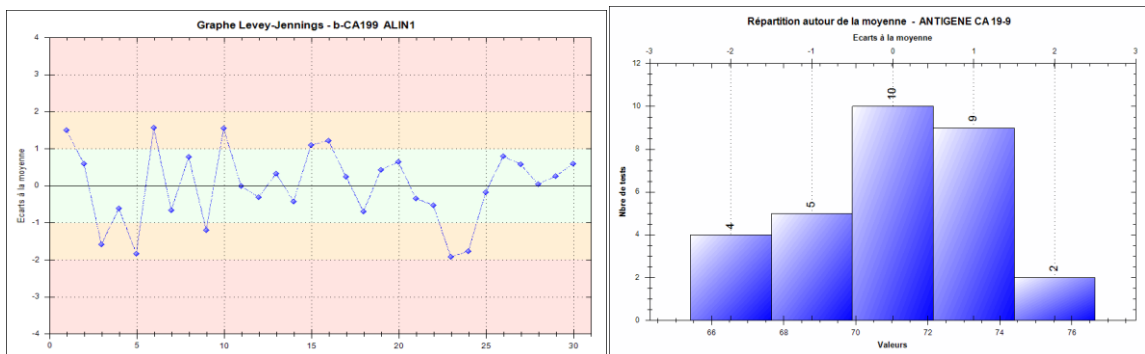


Figure 5 Medium Level of Reproducibility: Levey Jennings graph and the distribution around the mean –CA 19-9.

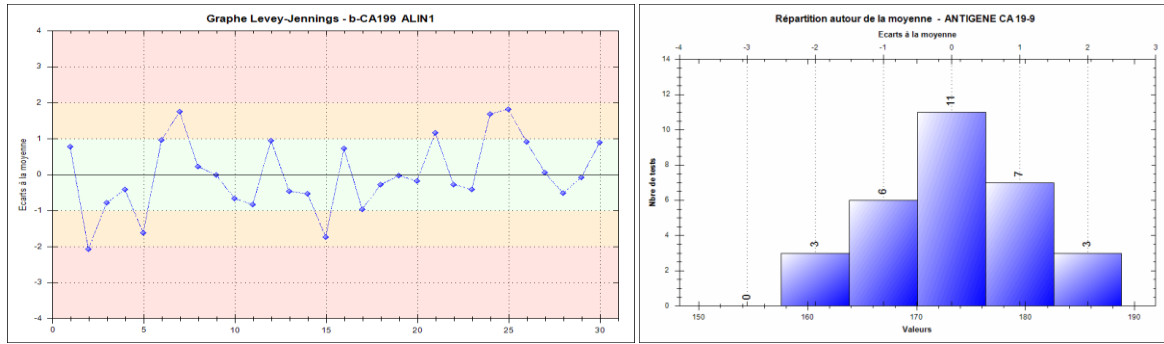


Figure 6 High Level of Reproducibility: Levey Jennings graph and the distribution around the mean –CA 19-9.

Table 2 Reproducibility results of blood assay of CA 19-9(Alinity ci) by level with comparison to FSBC and RICOS data (with expansion coefficient k = 1.211)

| Level of IQC | Number of values | Mean (UI/ml) | Standard Déviation | Coefficient of Variation CV (%) | Reference CV: FSBC 1999 (%) | Reference CV: RICOS (%) |
|--------------|------------------|--------------|--------------------|---------------------------------|-----------------------------|-------------------------|
| Low | 30 | 36.68 | 1.459 | 3.98 | 14.54 % | 9.69% |
| Medium | 30 | 71.03 | 2.248 | 3.16 | 12.11 % | 9.69 % |
| High | 30 | 173.2 | 6.25 | 3.61 | 12.11 % | 9.69 % |

4. Discussion

Repeatability and reproducibility, are statistical methods used in process control to measure the precision and variation present in our PLCs.

Repeatability evaluation determines optimum performance conditions and verifies the correct operation of the system, including instruments and reagents, for the parameter under evaluation.

Reproducibility evaluates the method's fidelity by varying factors such as operators, time, reagent batches and calibrations.

We chose CA19-9 as a control parameter in view of its value in the diagnosis and management of cancer diseases, particularly digestive and pancreatic cancers. The association between decreased CA19-9 and superior pancreatic cancer survival appears to be pronounced in patients with low platelet levels. This discovery could provide support for the underlying mechanisms of CA19-9 involved in platelet/cell interaction. To date, CA19-9 is a widely studied biomarker for the diagnosis and prognosis of pancreatic cancer (6).

This marker can also be used to guide treatment and management strategies for cancer patients as it is widely used in pancreatic cancer patients. CA 19-9 results should not be used on their own but in conjunction with a range of clinical, biological and radiological arguments, particularly with regard to screening (4), its sensitivity is around 80% and its specificity is higher (around 90%), with an upper limit of 37 U/ml; the threshold value. CA 19-9 is also widely used to predict the non-resectability of pancreatic adenocarcinoma (blood levels above 1000 U/ml) (7).It is also used for early detection of cancer recurrence. It is recommended that CA 19-9 concentrations be monitored using the same technique and in the same laboratory. Despite these drawbacks (false negatives in Lewis-negative subjects and false positives in benign pathologies, no tumor marker has superseded CA 19-9, which is still the best blood marker used in pancreatic cancer (8).

The Biochemistry Laboratory of the hospital university Mohammed VI of Oujda represents a leading healthcare facility in the eastern region of Morocco that is committed to maintaining the highest standards of analytical performance in order to provide reliable and accurate laboratory results for patients, following pre-established guidelines and

references [10-15]. As part of its commitment to quality, the central laboratory has set up a method verification procedure in accordance with Scope A, as well as an accreditation process.

We have followed a "Scope A" verification process, which is specifically designed for methods that have already been validated in their respective fields.

Abbott's biochemistry techniques are CE marked and essential for clinical diagnosis in medical laboratories.

These techniques are classified in the flexible category of scope A for method verification, as specified in COFRAC guide SH-GTA-04. The CE marking of Abbott's biochemical techniques guarantees their compliance with regulatory standards applicable to clinical diagnosis in medical laboratories [10-11].

The results of the repeatability and reproducibility study for CA 19-9 show satisfactory performance in relation to supplier data and CSFB criteria. [10-12].

Verification of the analytical performance of the serum CA 19-9 assay is essential in clinical laboratories, given its critical role as a key parameter in medical practice.

Serum CA 19-9 levels are widely used in the diagnosis and monitoring of various cancerous pathologies, particularly in the digestive tract and especially in the pancreas.

Accurate and reliable measurement of serum CA 19-9 is a prerequisite for satisfactory management of the disease treatment planning and monitoring of patient response [6-7]. The verification process involves a comprehensive assessment of the assay's performance characteristics, including repeatability, reproducibility, sensitivity, specificity and linearity. This rigorous process establishes the reliability and credibility of the assay, ensuring that results are accurate, precise and consistent, and therefore valid and meaningful for patient management [11-13].

Verification of the assay minimizes the risk of errors and ensures reliable results. The repercussions of inaccurate serum CA19-9 measurement can lead to diagnostic errors or misinterpretation of therapeutic follow-up, resulting in incorrect treatment plans. [12]. In addition, verification of the serum CA 19-9 assay is essential to comply with regulatory requirements and accreditation standards.

Compliance with these standards is essential to maintain the integrity of laboratory tests and guarantee the validity and reliability of patient results.

Verification of the serum CA 19-9 assay is necessary to maintain the credibility and competence of the laboratory.

Clinical laboratories must adhere to the highest standards of quality and accuracy in their testing processes to provide accurate and reliable results to clinicians and patients, in order to maintain healthcare providers' trust in laboratories [11-15].

5. Conclusion

Our study showed satisfactory results, meeting the criteria set by the supplier and the SFBC protocol. The Alinity ci demonstrated reliable analytical performance for the precise determination of CA 19-9, a valuable marker for the diagnosis and monitoring of cancer diseases. Reliable results from CA 19-9 analyses are essential for the management and follow-up of cancer patients, particularly those with digestive and pancreatic diseases. Compliance with the ISO 15189 method verification standard ensures accurate and reliable laboratory results, and reinforces its credibility. The verification process, which forms the basis of accreditation, enhances the quality of patient care and strengthens trust between patients and healthcare providers.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare no conflict of interest.

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