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(RESEARCH ARTICLE)

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Verification of analytical performance of Creatine Kinase isoenzyme MB (CK-MB) on the Alinity c® Experience from the Biochemistry Laboratory of Mohammed VI University Hospital in Oujda

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Abstract

The verification of analytical methods is a requirement of the NF EN ISO 15189 standard. It involves evaluating the performance of an analytical method according to a well-defined protocol and comparing it to pre-established analytical objectives. In this study, we present the results of the verification protocol for the measurement method of creatine kinase isoenzyme MB (CK-MB) on the Alinity c® analyzer, which is employed for functional exploration of the heart.

Our study is conducted according to the criteria of Scope A detailed in the Verification/Validation Guide for Methods in Medical Biology, following the SH GTA 04 recommendations from COFRAC. The verification focused on the CK-MB assay on the Alinity c® analyzer using International Federation of Clinical Chemistry (IFCC) method based on immunoinhibition principle for an assessment of analytical performance in terms of repeatability and intermediate precision. these results were aligned with the manufacturer's specifications.

The results obtained for various CK-MB assay verification criteria on the Alinity c® analyzer demonstrate satisfactory repeatability across one level with CV = 1.29%. Intra-laboratory reproducibility was satisfactory for one level with CV = 2.58%. The coefficient of variation (CV) values obtained in our study were compared with manufacturer's specifications, no CV reference values were established by French Society of Clinical Biology SFBC.

The findings from this study have allowed us to verify the performance of the CK-MB assay method and to compare them with the objectives set in the accreditation process to which our laboratory is committed.

Keywords: Method verification/validation; Repeatability; Reproducibility; Coefficient of variation; Alinity c®

1. Introduction

Creatine Kinase (CK) is an enzyme expressed in high amounts in muscle tissues, with three isoenzymes: CK-MB (creatine phosphokinase myocardial band), CK-MM (creatine phosphokinase skeletal muscle), CK-BB (creatine phosphokinase brain band) [1].

CK-MB is primarily associated with heart muscle cells but can also be present in skeletal muscles. The levels of CK-MB rise when there is damage to heart muscle cells. This increase can be detected between four to eight hours after chest pain begins, reaches its highest level within 18 to 24 hours, and goes back to normal within to 48 hours. High levels of CK-MB are strongly indicative of a heart attack (myocardial infraction) [2].

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Amongst advances in laboratory methodologies, the application of immunoinhibition technology in CK-MB assays has emerged as a promising avenue, offering increased sensitivity and accuracy in the detection of CK-MB levels. In this work, we present the results of a protocol for verifying the analytical performance of CK-MB assay method using an Abbot kit on the Alinity c® automated system in the biochemistry laboratory of the Mohammed VI University Hospital of Oujda.

1.1. Interest of Creatine phosphoKinase Myocardial Band (CK-MB) Determination

The Creatine Kinase Myocardial Band (CK-MB) test, being widely accessible, plays a crucial role in areas with limited resources globally. An increase in CK-MB levels has been linked to a greater risk of death in patients experiencing Acute Myocardial Infraction (AMI) [3]. suggesting that this biomarker may offer better prognostic insights than cardiac troponin [4]. Additionally, for patients who have undergone Percutaneous Coronary Interventions (PCI), a higher CK-MB level is associated with an increased risk of mortality during follow-ups at three months, six months, and one year. However, the emergence of cardiac markers with improved diagnostic capabilities has somewhat eclipsed the exploration of CK-MB's prognostic value, especially in developing countries where its affordability makes it a preferred option [5,6].

1.2. Principle of the assay method

Creatine kinase catalysis the reaction between creatine phosphate and ADP to form creatine and ATP. In the presence of glucose and hexokinase (HK), the ATP formed is converted into ADP and glucose-6-phosphate. The glucose-6-phostapte formed in the presence of glucose-6-phosphate dehydrogenase (G6P-DH) reacts with β-NADP + to form 6-phosphogluconate and β-NADPH. The presence of mouse antibodies that inhibit the activity of CK-MM by in the reaction mixture allows determination of the residual activity of the CK-B isoenzymes (CK-MB and CK-BB).

The activity of the CK-MB isoenzyme is calculated by multiplying the activity of CK-B by 2. Measurement of the change in absorbance due to transformation of β-NADP + into β-NADPH, carried out after a given time at determined time at 340 nm, is used to calculate the residual activity in the sample analyzed.

- Methodology: IFCC method based on immunoinhibition principle

2. Material and methods

This prospective study was conducted in the biochemistry laboratory of Mohammed VI University Hospital over a 30 days duration. It aimed to evaluate the reproducibility and repeatability of CK-MB measurements.

The research unfolded in two phases. Initially, to ascertain reproducibility, daily control tests at a single level were conducted over a 30 days period to verify consistency. The second phase involved gathering a variety of serum samples with different CK-MB concentrations to cover the full range of possible measurements. For each of these samples, 30 duplicate tests were carried out to assess repeatability.

The analysis was performed using a CK-MB reagent kit on the chemistry module, and data statistical analysis was facilitated by the EVM intermediate module from BYG Informatics. The coefficient of variation (CV) values obtained were compared with 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

In the reproducibility test, also called intermediate fidelity, a single sample is analyzed under different conditions to evaluate how variations in factors like timing, batches of reagents, operators and calibration processes affect the outcomes. The goal is to set acceptance criteria for prior information, especially within decision support systems.

The reproducibility outcomes were acceptable across one single level with coefficient of variation (CV) of 2.58%. These results are visually represented on the Levey-Jennings graphs, to improve the clarity of the results (Figure 1).

The intermediate fidelity CV is satisfactory, remaining below the established limits set by the manufacturer's specifications. The obtained CV values are then compared against these predefined limits, as outlined in Table 1.

Table 1 Reproducibility results of blood assay with comparaison to the manufacturer's specifications

Control Name	Number of values	Mean (UI/L)	Standard Deviation	Coefficient of Variation CV (%)	Reference CV: Manufacturer's specifications
Specific CK-MB control	30	49.20	1.270	2.58%	2.7%

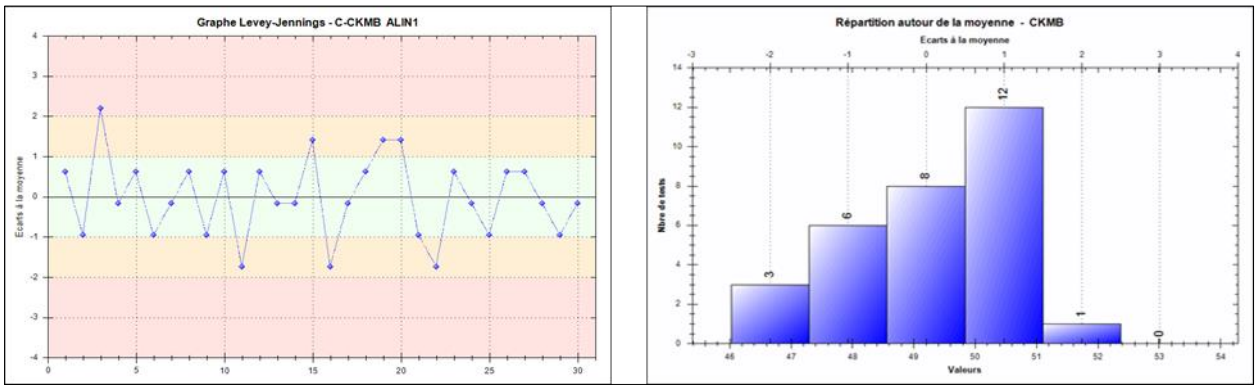

Figure 1 Reproducibility results: Levey Jennings Analysis: Distribution around the Mean - Regenerated by EVM

3.2. Repeatability results

Repeatability is evaluated by conducting multiple assays on identical samples by the same operator, ensuring uniformity in all measurement conditions, including reagents, instruments, calibration, and the operator, over the shortest feasible period. The findings from the verification criteria for the CK-MB assay reveal satisfactory repeatability, with a coefficient of variation (CV) of 1.29% across 30 samples (Figure 2).

The conclusion asserts that the CV of repeatability is accurate and remains below the tolerated limit. Similar to the intermediate fidelity results, the manufacturer's limits with expansion factors are referenced. The CV values are then compared to these limits (Table 2).

Table 2 Repeatability results of blood assay with comparaison to manufacturer's specifications

Control Name	Number of values	Mean (UI/L)	Standard Deviation	Coefficient of Variation CV (%)	Reference CV: Manufacturer's specifications
Specific CK-MB control	30	56.50	0.731	1.29%	2.4%

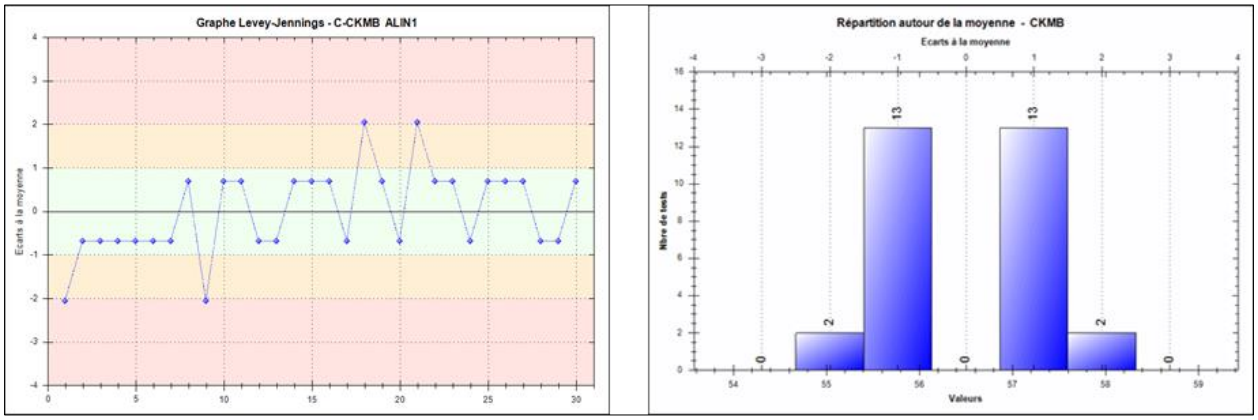

Figure 2 Repeatability results: Levey Jennings Analysis: Distribution around the Mean – Regenerated by EVM

4. Discussion

CK-MB is an indispensable biomarker in the diagnosis and management of acute myocardial infarction and other cardiac conditions. Its role in energy metabolism in cardiac cells and its release pattern during myocardial injury make it a key indicator of cardiac health, particularly in the early stages of cardiac injury. With advancements in diagnostic technology, the measurement of CK-MB has become more precise, contributing to improved patient care in cardiac diagnostics. As research continues, the understanding and application of CK-MB in clinical practice will likely evolve offering new insights into cardiac diagnostics and treatment strategies, Furthermore, the measurement of CK-MB can assist in assessing the size of a myocardial infarction and monitoring the efficacy of reperfusion therapy. The reappearance or secondary rise in CK-MB levels can indicate additional myocardial damage or a complication of the initial infarction [7,8].

The laboratory assesses the performance levels achieved through its method by comparing these results with expected reference data from sources such as suppliers or scholarly societies. Based on this comparison, it concludes whether its method meets the necessary criteria for the specific tests conducted. The specifications of the samples used in this process are clearly defined, ensuring the validity and relevance of the comparison [9].

This study aimed to verify the analytical performance of creatine kinase isoenzyme MB (CK-MB) assays on the Alinity c® system within the Biochemistry Laboratory of Mohammed VI University Hospital in Oujda. CK-MB holds paramount importance in the biochemical diagnosis and monitoring of acute myocardial infarction (AMI), making its accurate and reliable detection crucial for patient care. The analytical performance of CK-MB assays can significantly influence clinical decision-making, particularly in the early stages of AMI where timely intervention can drastically alter patient outcomes, treatment planning and monitoring of patient response.

Statistical methods such as repeatability and intermediate fidelity are crucial in maintaining accuracy within automated laboratory systems. The intermediate fidelity test, also known as intralaboratory reproducibility, involves analyzing a single sample under different conditions, including changes in operators, time, reagent batches, and calibration processes. This approach helps in establishing acceptance criteria that consider biological variation, which is essential for decision support systems. It enables the objective interpretation of results, ensuring that decisions based on these results are reliable and informed [10].

The repeatability assessment has shown that the immunoinhibition CK-MB assay used in our laboratory exhibits exceptional precision. This is evidenced by a consistently low coefficient of variation for repeatability, indicating minimal variability in repeated measurements under the same conditions. These results highlight not only the method's reliability but also its stability and robustness. The coefficients of variation derived from the analysis of repeatability and intermediate fidelity were well within acceptable limits, meeting the supplier's specified criteria. These outcomes confirm the efficacy of the IFCC method based on immunoinhibition principle for CK-MB determination on the Abbott Alinity CI analyzer, demonstrating its consistency and stability. The congruence of these findings with the manufacturer's specifications further attests to the method's robustness and reliability, marking it as suitable for precise and reliable measurements in critical clinical diagnostics.

5. Conclusion

In summary, our findings suggest that the Alinity c® system's CK-MB assay exhibits a high degree of accuracy and precision, consistent with the manufacturer's specifications. By conducting thorough validation against established standards and detailed assessments of repeatability and intermediate fidelity, we have proven the robustness and precision of our approach. These findings within the Biochemistry Laboratory of Mohammed VI University Hospital in Oujda underscores the importance of continuous technological advancement and rigorous performance validation in clinical biochemistry. As we move forward, such endeavors will remain pivotal in advancing the quality of patient care and outcomes in the face of evolving clinical challenges.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare no conflict of interest.

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