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(RESEARCH ARTICLE)

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Verification of the high-sensitivity troponin I assay method on Abbott Alinity I: Experience of the central Biochemistry Laboratory of Ibn Sina University Hospital in Rabat

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Abstract

Verification of an analytical method in clinical laboratories is an essential process under the responsibility of biologists. It consists in assessing the analytical performance of a method and/or technique for assaying a biological parameter, in order to guarantee accurate and reliable results that benefit both the patient and the prescriber. The aim of our work, carried out in the Central Biochemistry Laboratory of the Ibn Sina University Hospital in Rabat-salé, is to verify the Troponin I (cTnI) assay « Range A » on Abbott's Alinity i automated system, using the chemiluminescent microparticle immunoassay (CMIA) method. The methodology adopted was based on the recommendations of the COFRAC (Comité Français d'Accréditation) accreditation technical guide, involving evaluation of the main analytical performances: repeatability, reproducibility and method comparison, plus an external quality assessment (EQA) to measure accuracy. The results obtained from this evaluation were compliant, and the CVs (coefficients of variation) produced were compliant and satisfactory with the supplier's data and with the Ricos learned society.

Keywords: Troponin I; Method Verification; Analytical performance; External Quality Evaluation; Alinity I

1. Introduction

Method verification in medical biology laboratories is a process that consists of evaluating the analytical performance of a method or technique for measuring a biological parameter, aiming to guarantee precise and reliable analytical results as well as the relevance of clinical interpretations beneficial to the patient and the prescriber.

This is a permanent commitment of biologists and an essential step as stipulated in the quality standards, in particular the Guide for the Good Execution of Medical Biology Analyses (GBEA) applicable in Morocco since November 2011 to all laboratories, both private and public (1), ISO 15189 standards, learned societies (RICOS, SFCB, etc.) and the technical guide of COFRAC (French accreditation committee) (2,3,4).

Troponin (Tn) is a structural protein of skeletal and cardiac muscle myofibrils. Troponin I (cTnI) measurement uses immunometric techniques with cardiospecific antibodies. It is the most sensitive and specific biomarker of myocardial damage. It currently represents the gold standard for early or retrospective diagnosis of myocardial infarction (MI) and the prognostic marker of unstable angina (5,6).

The objective of our study is the verification of a "scope A" dosage method, involving a study of the analytical performance of Troponin I (cTnI) dosage on "Alinity i" Abbott, by a chemiluminescence microparticle immunological

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method (CMIA), within the Central Biochemistry Laboratory of the Ibn Sina University Hospital of Rabat-Salé where the entire study was carried out, during a renewal of high-flow analytical equipment.

2. Material and methods

2.1. Biological principle of dosage

The Abbott "Alinity i" Troponin I (cTnI) assay is based on a two-step immunological technique for the quantitative measurement of cTnI in human plasma and serum using chemiluminescence microparticle immunoassay (CMIA) technology.

The sample and the paramagnetic microparticles coated with anti-troponin-I antibodies are brought together and incubated.

Cardiac troponin-I present in the sample adheres to microparticles coated with anti-troponin-I antibodies. The mixture then undergoes a washing process. The acridinium-marked anti-troponin-I antibody conjugate is added to form a reaction mixture and then incubated.

After another wash cycle, the preactivation and activation solutions are added. The resulting chemiluminescent reaction is measured in relative light units (URL).

There is a direct relationship between the amount of Troponin present in the sample and the URLs detected by the optical system.

2.2. Dosing technique:

The Troponin I assay was performed using a dedicated reagent kit (STAT High sensitive Troponin-I Reagent kit) on the immunology module of the Abbott Alinity i analyzer (code G73445R04, Lot 40058UD00-00193, Ref: 08P1332 / 08P1322) consisting of:

- **Microparticles:** coated with anti-troponin-I antibodies (mouse, monoclonal) in TRIS buffer with a protein stabilizer (bovine).
- **Conjugate:** Acridinium-marked anti-troponin-I antibodies (mouse/human chimeric monoclonal) in MES buffer with protein stabilizer (bovine) and human IgG.

Data processing was carried out via Middleware EVM (BYG Informatique), serving as intermediary software between the Alinity platform and the biological validation software "Elabs" in order to obtain the different equations necessary for completing the verification file. The CVs (coefficient of variation) obtained were evaluated and compared to the CVs set by the learned society of RICOS (2014).

2.3. Description of the method

Detailed description of the verification/validation method for Troponin I dosage, in accordance with document SH FORM 43 as part of its adaptation to the process approach of version 2022 of the ISO 15189 standard:

Description of the method	
Analyte/Measurand	**Troponin-I Assay**
Principle of measurement	Chemiluminescence microparticle immunoassay (CMIA).
Method of measurement	Alinity i STAT High Sensitive Troponin-I Reagent Kit (also called hsTnI Risk Strat)
Primary sample type	Serum or plasma
Container type	- Serum with and without separator. - Serum with thrombin-based clot activator. - Lithium heparin with and without separator. EDTA K2 EDTA K3
Sample pretreatment	Transport to the laboratory: - Less than 8 hours: Store the primary tube at room temperature.

	- More than 24 hours: Store 2 ml of serum at 2 to 8°C. Centrifugation: - Serum samples: Centrifuge at 3000 to 3500 x g for 30 minutes. - Plasma samples: Centrifuge at 13 000 to 13 500 x g for 30 minutes. For frozen samples: - Thaw completely - Homogenize by vortexing at low speed or inverting 10 times. - Transfer the clarified sample to a sample cup.
Unit	- Default: ng/L - Other units: ng/mL, µg/L, pg/mL
Interpretation criteria	The measuring range of the Alinity i STAT High Sensitive Troponin-I assay is 10 to 50,000 pg/mL (0.01 to 50 ng/mL)
Instrument	Alinity i
Reagent reference	STAT High Sensitive Troponin-I Reagent Kit, REF 08P1327 /08P1337
Calibration material	08P1301 Alinity i STAT High Sensitive Troponin-I Calibrators
Calibration type, number of levels and values	4 parameter logistic curve (4PLC, Y-weighted)

2.4. Analytical performance

As part of method verification/validation, we have adopted strategy 1 or scope A (4), which consists of verifying the performance announced by the supplier or desired by the laboratory when implementing a new analysis method, in particular: reproducibility, repeatability, method comparison, accuracy.

2.4.1. Intermediate fidelity study (reproducibility)

The reproducibility test is an essential step in the analytical performance evaluation process. It allows the same sample to be analyzed, in the same laboratory, on the same instrument, using the same method but with different environmental conditions: possible variation in the batch of reagents, in the calibration, by different operators (4). It allows the acceptance criteria for the differences observed between the results obtained and the pre-established compliance limits to be determined by comparing them with clinical requirements.

In our study, the reproducibility assessment was carried out through the daily passage of three levels of Abbott® controls: low, medium and high. The reproducibility study was carried out on two different batches of reagents. The main objective was to evaluate the consistency and reliability of the test.

The data allows the calculation of the mean, CV and standard deviation. The CVs calculated for each concentration level are compared with the CVs announced by the learned society of RICOS (2014).

2.4.2. Repeatability study

Repeatability consists of dosing the same sample, by the same operator, under the same operating conditions, same environment, for all measurements, and for a short period. Repeatability study is an essential step when setting up a new automaton to know the initial performance of the system (reagent/instrument) and verify its correct functioning (4).

In our study, the assessment of repeatability (intra-series imprecision) was carried out by measuring troponin I 20 times while respecting the above conditions, for the three control levels: low, medium and high.

The data allows the calculation of the mean, CV and standard deviation. The CVs calculated for each concentration level are compared with the CVs announced by the learned society of RICOS (2014).

2.4.3. Method comparison study:

Comparing an analysis method involves evaluating the results of patient samples (chosen to cover the pathophysiological extent) obtained by two different methods and highlighting differences that may affect the interpretation of the results.

Our comparative study between Alinity ® and Architect i2000® was carried out on 22 patient samples, covering the entire measurement range (0.04 to 50 ng/ml). Each sample is processed in both machines.

The results obtained by the old "Architect i2000" automaton are noted Xi and those of the new "Alinity i" automaton are noted Yi, thus allowing the determination of the linear regression line, the correlation coefficient and to draw the Bland and Altman diagram.

2.4.4. Accuracy

The accuracy or closeness of agreement between the measured value and the true value of a measurand should be studied from a reference value obtained from a reference method. However, to date, medical biology laboratories evaluate their accuracy based on the results of external quality assessments (EQA) or the exchange of inter-laboratory results of samples (4).

The Central Biochemistry Laboratory of the Ibn Sina University Hospital of Rabat-Salé is engaged in a BIO-RAD EQAS program (cycle 11) covering the period from 18 April 2022 to19 March 2023, allowing an External Quality Evaluation (EQE) of the dosage of cardiac markers, in particular Troponin I. The accuracy study corresponds to a comparison of the blind dosage results of Troponin I of the 12 unknown samples assayed in our laboratory during the study period compared to the «peer group» as well as «all techniques».

3. Results

3.1. Intermediate fidelity results (reproducibility)

Table 1 Results of the Troponin-I reproducibility study on « Alinity i »

Samples	**Number of values (N)**	**mean (ng/ml)**	**Standard deviation (ng/ml)**	**CV (%)**	**CV (%) Ricos**	**CV (%) Ricos extended (K=1.418)**
Level 1 (low)	7	15.05	0.77	**5.15**	7.02	**10.17**
Level 2 (medium)	8	51.41	3.19	**6.20**	7.02	**9.95**
Level 3 (high)	8	907.20	16.38	**1.80**	7.02	**9.95**

The reproducibility results for all low, medium and high levels, giving coefficients of variation (CV1, CV2 and CV3) of 5.15%, 6.20% and 1.80% respectively (Table 1).

These results were presented graphically using Levey-Jennings charts to further illustrate the results obtained (Figure 1, 2 and 3).

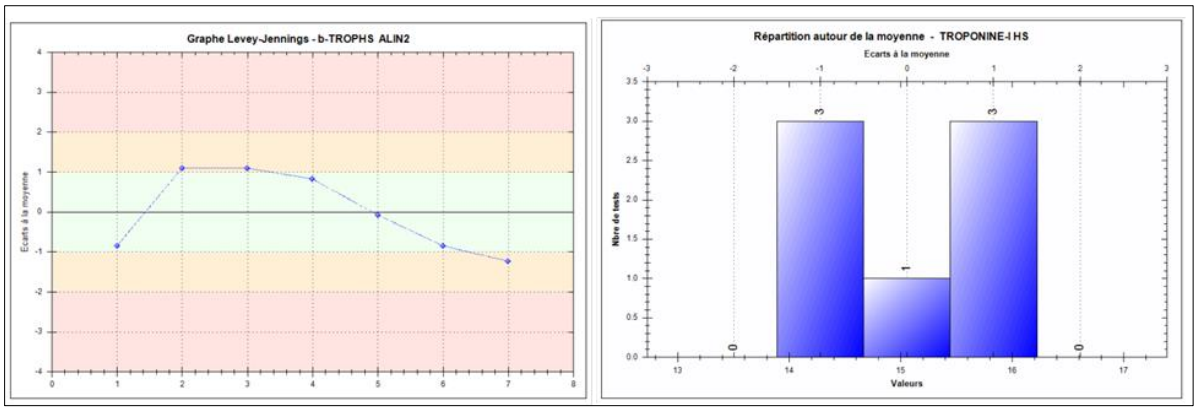

Figure 1 Representation of reproducibility results (low level) : Levey-Jennings curve and distribution around the mean

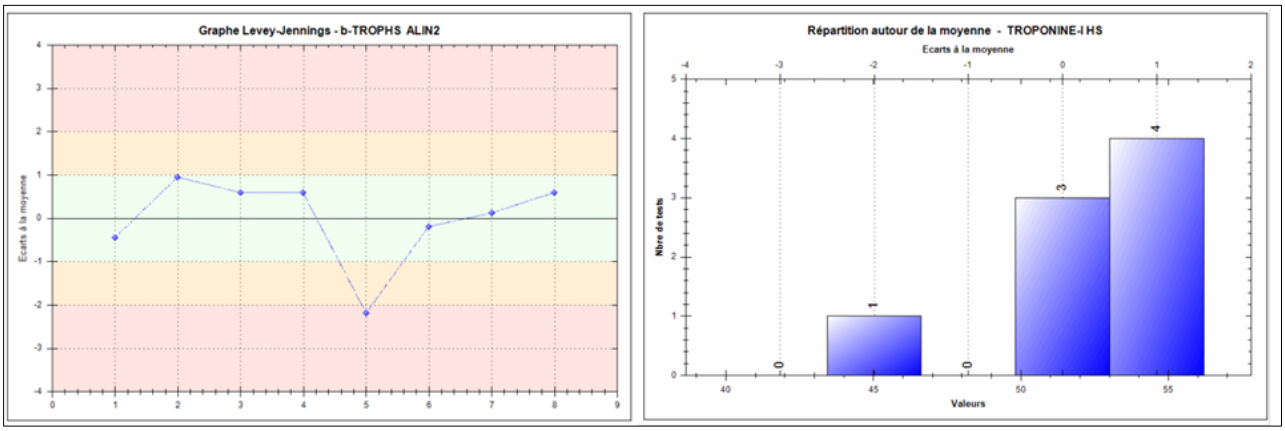

Figure 2 Representation of reproducibility results (medium level): Levey-Jennings curve and distribution around the mean

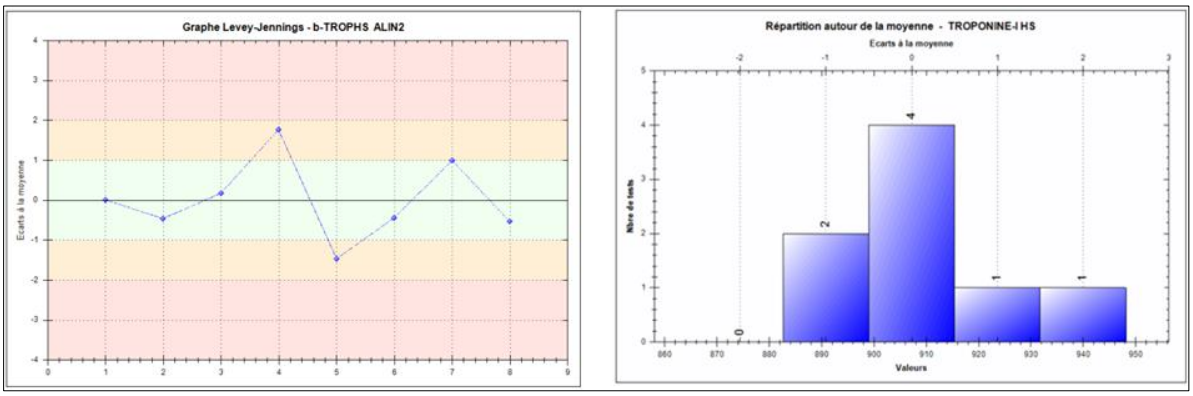

Figure 3 Representation of reproducibility results (high level) : Levey-Jennings curve and distribution around the mean

3.2. Repeatability results

The results of the repeatability test for all low, medium and high levels, giving coefficients of variation (CV1, CV2 and CV3) of 3.02%, 2.49% and 1.65% respectively (Table 2).

These results were presented graphically using Levey-Jennings charts to further illustrate the results obtained (Figure 4, 5 and 6).

Table 2 Results of the Troponin-I repeatability study on « Alinity i »

Samples	Number of values (N)	mean (ng/ml)	Standard deviation (ng/ml)	CV (%)	CV (%) Ricos	CV (%) Ricos extended (K=1.260)
Level 1 (low)	20	14.57	0.44	3.02	5.27	6.63
Level 2 (medium)	20	49.19	1.223	2.49	5.27	6.63
Level 3 (high)	20	886.14	14.61	1.65	5.27	6.63

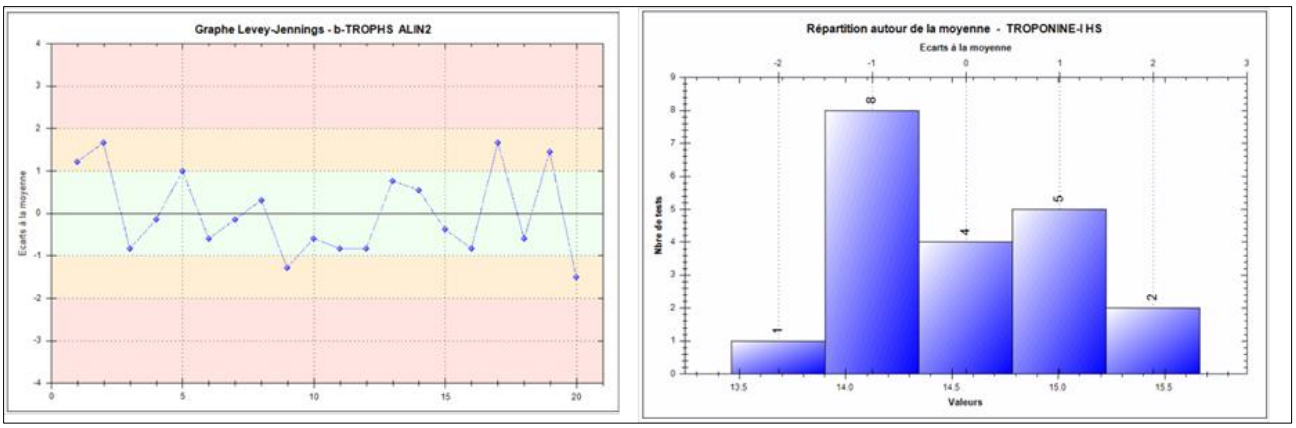

Figure 4 Representation of repeatability results (low level) : Levey-Jennings curve and distribution around the mean

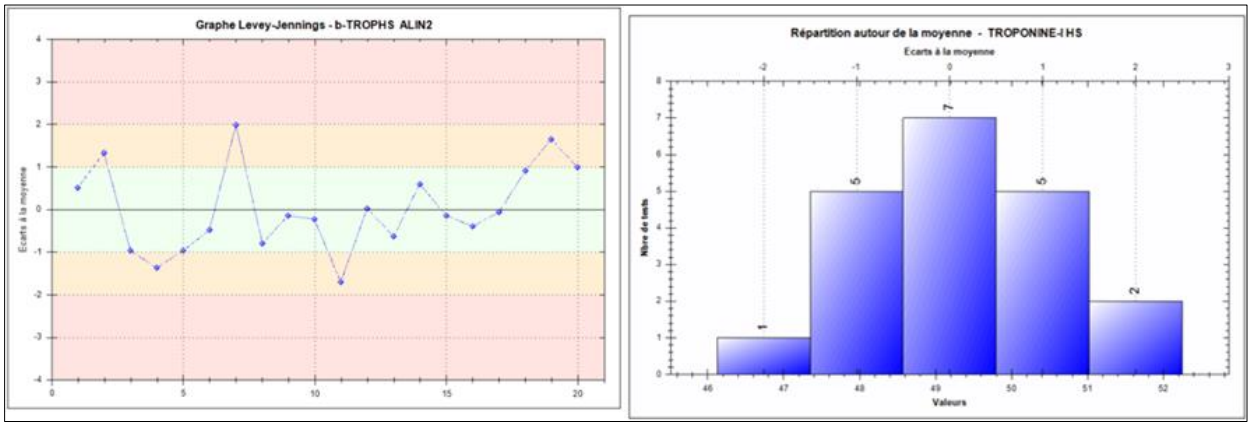

Figure 5 Representation of repeatability results (medium level) : Levey-Jennings curve and distribution around the mean

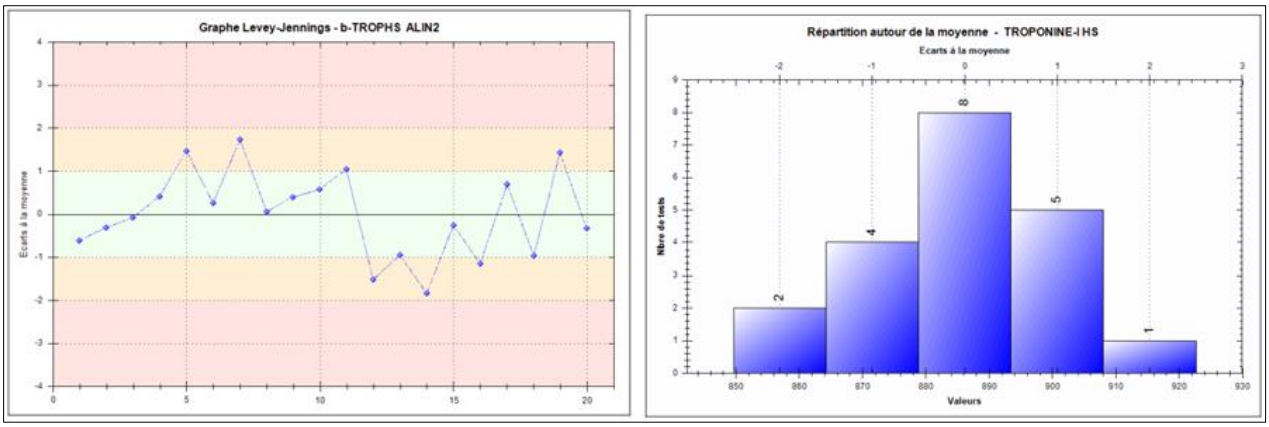

Figure 6 Representation of repeatability results (high level) : Levey-Jennings curve and distribution around the mean

3.3. Method comparison results

The results of the comparative study between Alinity® and Architect i2000® were analyzed by determining the linear regression line, defined by the equation Y = 0.94X - 0.17, with a correlation coefficient of 1.00 (Figure 7). In addition, the Bland and Altman concordance assessment method was performed, comparing the means of the measurements (abscissas) with their differences (ordinates) (Figure 8).

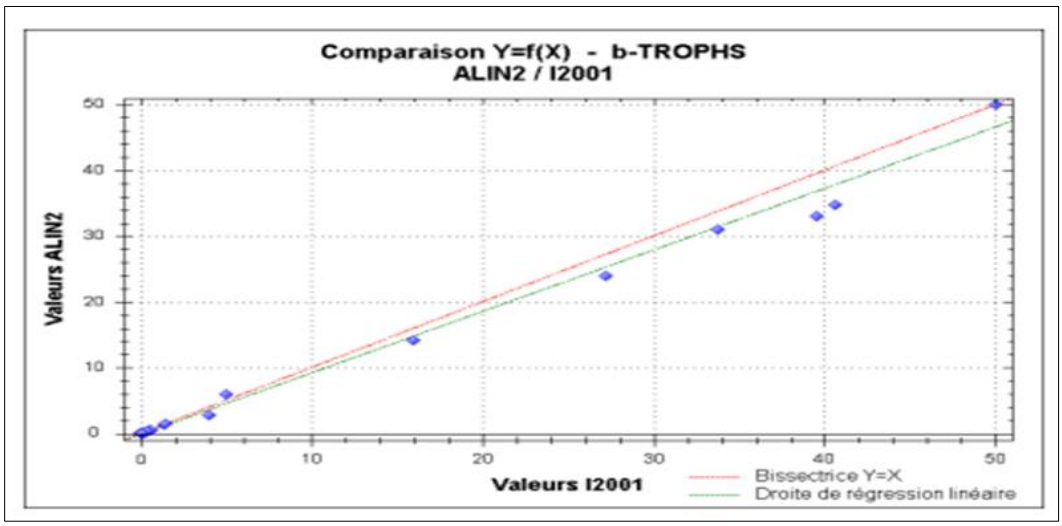

Figure 7 Troponin I correlation study : Comparison between values obtained on Alinity® and Architect i2000®

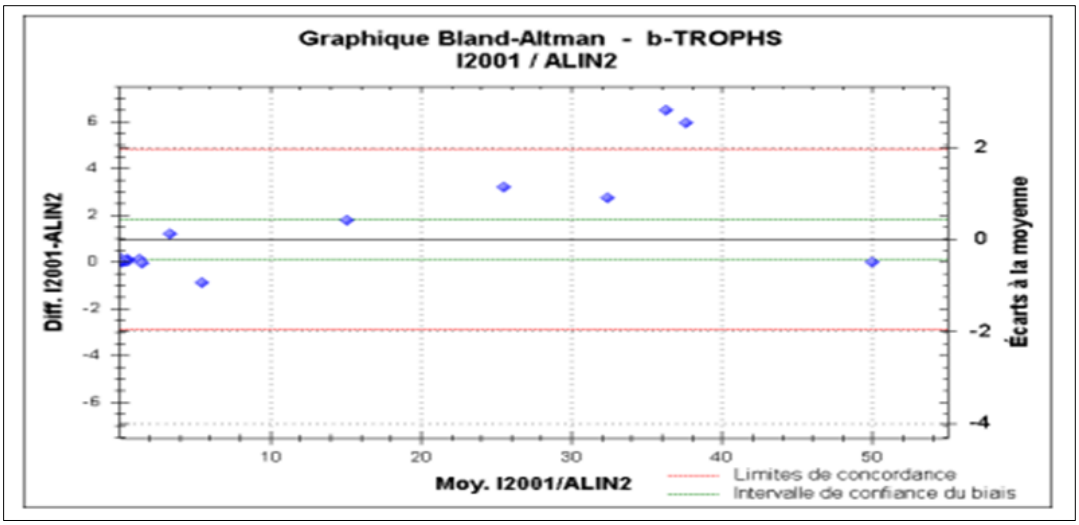

Figure 8 Bland-Altman diagram

3.4. Accuracy results

The results of the external quality assessment (EQA) are presented in table 3:

Table 3 Results of the external quality assessment

Sample	Number (N)	Lab Value (ng/l)	Target (peer group)	Target (all techniques)	Bias (%) / (peer group)	Bias (%) / (all techniques)	Bias (%) optimal limit (EFLM)(*)
Cycle 11 sample 1	78	2240	2234	2315	- 0.10	- 0.10	13.96
Cycle 11 sample 2	101	980	981	1017	- 0.10	- 0.10	
Cycle 11 sample 3	109	54.1	56.2	58.4	- 3.72	- 7.34	
Cycle 11 sample 4	102	232.19	254	266	- 8.73	- 12.80	
Cycle 11 sample 5	107	2253.32	2283	2371	- 1.30	- 4.94	
Cycle 11 sample 6	114	250.69	258	267	- 2.85	- 6.08	
Cycle 11 sample 7	114	922.56	991	1028	- 6.89	- 10.30	
Cycle 11 sample 8	117	2132.97	2267	2342	- 5.90	- 8.92	
Cycle 11 sample 9	117	56.01	55.3	57.6	1.22	- 2.60	
Cycle 11 sample 10	107	263.65	256	265	3.04	- 0.39	
Cycle 11 sample 11	125	899.3	965	1018	- 8.71	- 11.60	
Cycle 11 sample 12	121	56.45	54.2	56.9	4.11	- 0.87	

(*) : European Federation of Clinical Chemistry and Laboratory Medicine

4. Discussion

Verification/validation of methods within a medical biology laboratory is a crucial and indispensable process that ensures accurate and reliable results. It is a regulatory requirement set out in GBEA and ISO 15189 (6,7).

Within the framework of the NF EN ISO 15189 and 22870 standards, the medical biology laboratory must verify that its dosage methods are valid and mastered within the laboratory, and that they meet the needs of patients and prescribers.

In our study, we carried out an on-site "scope A method verification" of the analytical performance of Troponin I Hs dosage on Alinity i from Abbott® during a renewal of analytical equipment at the Central Biochemistry Laboratory of Ibn-Sina University Hospital in Rabat-Salé. The most relevant modules of this verification were detailed in the results section of this work. A comparison of the results obtained with respect to the acceptable limits previously defined by learned societies (Ricos) and recently conducted studies on Troponin dosage allows for a critical reading and a correct interpretation of the clinical impact of the results.

4.1. Intermediate fidelity (reproducibility):

The results of the study of the reproducibility of the Troponin I dosage on Alinity i are consistent and satisfactory with the supplier's data and the Ricos learned society (Table 4).

Table 4 Results of the Troponin-I reproducibility study with comparison to RICOS data

Samples	Number of values (N)	mean (ng/ml)	Standard deviation (ng/ml)	CV (%)	CV (%) Ricos	CV (%) Ricos extented (K=1.418)	Conclusion
Level 1 (low)	7	15.05	0.77	5.15	7.02	10.17	Compliant
Level 2 (medium)	8	51.41	3.19	6.20	7.02	9.95	Compliant
Level 3 (high)	8	907.20	16.38	1.80	7.02	9.95	Compliant

4.2. Repeatability

The results of the repeatability study of the Troponin I assay on Alinity i are consistent with the supplier's data and the Ricos learned society (Table 5). The coefficients of variation (CV) obtained are satisfactory and appear to be consistent with those of the study mentioned by Westwood et al (8). Thus, it is concluded that the precision is consistent with the requirements set for the troponin I assay on Alinity i.

Table 5 Results of the Troponin-I repeatability study with comparison to RICOS data

Samples	Number of values (N)	mean (ng/ml)	Standard deviation (ng/ml)	CV (%)	CV (%) Ricos	CV (%) Ricos extented (K=1.260)	Conclusion
Level 1 (low)	20	14.57	0.44	3.02	5.27	6.63	Compliant
Level 2 (medium)	20	49.19	1.223	2.49	5.27	6.63	Compliant
Level 3 (high)	20	886.14	14.61	1.65	5.27	6.63	Compliant

4.3. Method comparison

Comparison of assay results with the STAT Troponin-I kit on the Architect i2000® also showed better clinical precocity of the Abbott® technique, particularly for low values. The choice of antibodies has a significant impact on assay performance due to the presence of different forms of Troponin in the bloodstream.

Regarding the detection limit, the literature reports a detection limit of 1.6 ng/L; which corresponds to the minimum concentration for which the coefficient of variation is less than 10%(9).

4.4. Accuracy

Our laboratory is engaged in an External Quality Assessment (EQA) program aimed at improving laboratory performance. According to the study by Ricos et al (10), the results of the participating laboratories in this assessment are compared against a specification based on biological variation (BV), which was described in 1999 and was modified in 2019, when the European Federation of Laboratory Medicine (EFLM) database was published. Our accuracy results (Table 6) were consistent with the «peer group» as well as «all techniques».

Table 6 Comparison of EQA results with the peer group as well as all techniques

ACCURACY (from external quality controls: EEQ)								
Sample	**Number (N)**	**Lab Value (ng/l)**	**Target (peer group)**	**Target (all techniques)**	**Bias (%) / (peer group)**	**Bias (%) / (all techniques)**	**Bias (%) optimal limit EFLM)(*)**	**Conclusion**
Cycle 11 sample 1	78	2240	2234	2315	- 0.10	- 0.10	13.96	Compliant
Cycle 11 sample 2	101	980	981	1017	- 0.10	- 0.10		Compliant
Cycle 11 sample 3	109	54.1	56.2	58.4	- 3.72	- 7.34		Compliant
Cycle 11 sample 4	102	232.19	254	266	- 8.73	- 12.80		Compliant
Cycle 11 sample 5	107	2253.32	2283	2371	- 1.30	- 4.94		Compliant
Cycle 11 sample 6	114	250.69	258	267	- 2.85	- 6.08		Compliant
Cycle 11 sample 7	114	922.56	991	1028	- 6.89	- 10.30		Compliant
Cycle 11 sample 8	117	2132.97	2267	2342	- 5.90	- 8.92		Compliant
Cycle 11 sample 9	117	56.01	55.3	57.6	1.22	- 2.60		Compliant
Cycle 11 sample 10	107	263.65	256	265	3.04	- 0.39		Compliant
Cycle 11 sample 11	125	899.3	965	1018	- 8.71	- 11.60		Compliant
Cycle 11 sample 12	121	56.45	54.2	56.9	4.11	- 0.87		Compliant

(*) : European Federation of Clinical Chemistry and Laboratory Medicine

5. Conclusion

Our study showed results deemed compliant and satisfactory, meeting the criteria set by the supplier and the learned society Ricos. Alinity i demonstrated accurate and precise analytical performance for the dosage of Troponin I HS, a valuable cardiac marker for the diagnosis and monitoring of acute coronary syndrome (ACS).

The process of verifying the analysis method in accordance with ISO 15189 standard constitutes the basis of accreditation, thus guaranteeing an improvement in the quality of care and strengthening trust between patients, prescribers, and the medical analysis laboratory, thus ensuring rapid and effective care.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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