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Verification of analytical performance of cyclosporine assay on the Abbott Alinity ci® at the biochemistry laboratory of Mohammed VI university hospital Oujda

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Abstract

In medical laboratories, the verification of methods ensures precise measurements, complying with regulatory standards like the Moroccan Guide for Medical Laboratory Analysis (GBEA) and ISO 15189. After organ transplantation, blood levels of cyclosporine are often monitored closely, and its dosage adjusted accordingly to maintain therapeutic levels while minimizing the risk of toxicity. This study aims to verify the analytical performance of the cyclosporine assay on the Abbott Alinity ci® analyzer. Conducted at Mohammed VI university hospital's biochemistry laboratory over 30 days, it involved two phases: evaluation of reproducibility and repeatability. Reproducibility was assessed by daily testing of the control samples at three concentration levels (low, medium, and high), and repeatability was measured by subjecting each serum sample to 30 individual assay runs. Cyclosporine determination used a dedicated reagent kit on the Abbott Alinity ci® analyzer's immunology module, with data processing via the BYG middleware. The resulting coefficient of variation (CV) values were compared against standards set by the supplier. The investigation demonstrated a commendable repeatability across all concentration levels, with CV values of 3.59%, 3.23%, and 3.46% respectively. Intermediate fidelity examination yielded satisfactory results, with CV values of 3%, 4.83%, and 6.01% for low, medium, and high levels respectively. In conclusion, our study confirms the laboratory's capability to provide accurate and precise cyclosporine assay results for clinical use, meeting the recommended criteria set by the suppliers.

Keywords: Cyclosporine; Analytical performance; Repeatability; Reproducibility; Chemiluminescent immunoassay; Alinity ci® analyzer

1. Introduction

1.1. Overview of cyclosporine

Cyclosporine, an immunosuppressant introduced in the early 1980s, has significantly improved outcomes in organ transplantation by reducing rejection rates and enhancing graft survival. However, its narrow therapeutic index and considerable interpatient variability require blood levels monitoring in order to optimize efficacy and limit toxicity.(1)

Cyclosporine exerts its effect by inhibiting calcineurin, which in turn inhibits the synthesis of IL-2, an essential cytokine for T lymphocytes self-activation and differentiation, the major effector of chronic graft rejection.(2) For this reason, it's mainly used for post-transplant organ rejection prevention. Additionally, it can be used to treat a host of other indications, both FDA and non-FDA-approved, including some autoimmune conditions, such as rheumatoid arthritis, psoriasis, and GVHD to name a few.(3)

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1.2. Interest of cyclosporine dosage

On the other hand, cyclosporine use is commonly associated with several side effects. These range from mild gastrointestinal upset, gum hypertrophy, and hypertrichosis, to more serious disturbances, mainly hypertension, hyperlipidemia, and diabetes.(4) Of note, cyclosporine is notorious for its nephrotoxic effects due to increased tone of the glomerular afferent arteriole.(5)

Management of patients taking cyclosporine is further complicated by the significant inter- and intra-patient variability in pharmacokinetics and metabolism. With several factors affecting its blood levels, from food and drug interactions to genetic variants in cytochrome P450 enzymes.(4) Therefore, regular blood level monitoring is required to ensure therapeutic efficacy while limiting adverse reactions.(6)

Our study aims to evaluate the analytical performance of the serum cyclosporine assay method using an Abbott Alinity ci® automated system in the biochemistry laboratory of Mohammed VI university hospital in Oujda, Morocco.

1.3. Principle of cyclosporine assay method

This assay employs a two-step immunoassay technique to quantitatively measure cyclosporine levels in human whole blood, utilizing chemiluminescent microparticle immunoassay (CMIA) technology.

Initially, a manual pretreatment procedure is performed. This involves lysing the whole blood sample using a precipitation reagent and centrifuging the sample. The supernatant is decanted into a transplant pretreatment tube, then placed on the Alinity ci® analyzer.

Sample and anti-cyclosporine coated with paramagnetic microparticles are mixed and incubated. The cyclosporine within the sample adheres to the anti-cyclosporine-coated microparticles. Subsequently, the mixture undergoes a washing process. After that, an acridinium-labeled conjugate of anti-cyclosporine is introduced, creating a reaction mixture that is again incubated. Post a wash cycle, the addition of pre-trigger and trigger, then solutions follow. The resultant chemiluminescent reaction is gauged in relative light units (RLUs). The system optics detect RLUs, showing a direct correlation between the cyclosporine 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 30 days. The working methodology adopted is based on the recommendations issued by the French accreditation committee (COFRAC) using the accreditation technical guide GTA 04 protocol. 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. In the subsequent phase, for repeatability assessment, samples were categorized into three groups: low, medium, and high. Each sample underwent analysis 30 times under the following conditions: same operator, same batch of reagents, same instrument, same calibration on the same day.

2.1. Statistical processing of the data was carried out using the EVM intermediate module from BYG Informatics.

In the absence of specific guidelines or reference values from professional societies such as the French Society of Clinical Biochemistry (SFBC) or organizations like RICOS, our study adopted an alternative approach to assess the acceptability of coefficient of variation (CV) values for cyclosporine assays. Our approach entailed comparing the CV values obtained from our assays with the manufacturer's specifications or recommended performance criteria. These specifications, derived from the manufacturer's validation studies and quality control assessments, served as benchmarks for evaluating the precision and reliability of our assay. By aligning our CV values with the manufacturer's recommendations, we ensured that our assay performance met the established standards for accuracy and consistency.

3. Results

3.1. Repeatability results

The results obtained for the cyclosporine assay verification criteria show satisfactory repeatability for the 3 levels (1: low / 2: medium/ 3: high) with respectively CV1 = 3.59%, CV2= 3.23%, CV3= 3.46% (Table 1).

Table 1 Repeatability results of blood assay by level with comparison to the reference CV

Level of IQC	Numbers of value	Mean (ng/ml)	Standard deviation	Coefficient of variation CV (%)	Reference CV (%) set by the supplier
Low	30	118.15	4.245	3.59	4.8
Medium	30	486.15	15.685	3.23	4.5
High	30	713.19	24.695	3.46	4.4

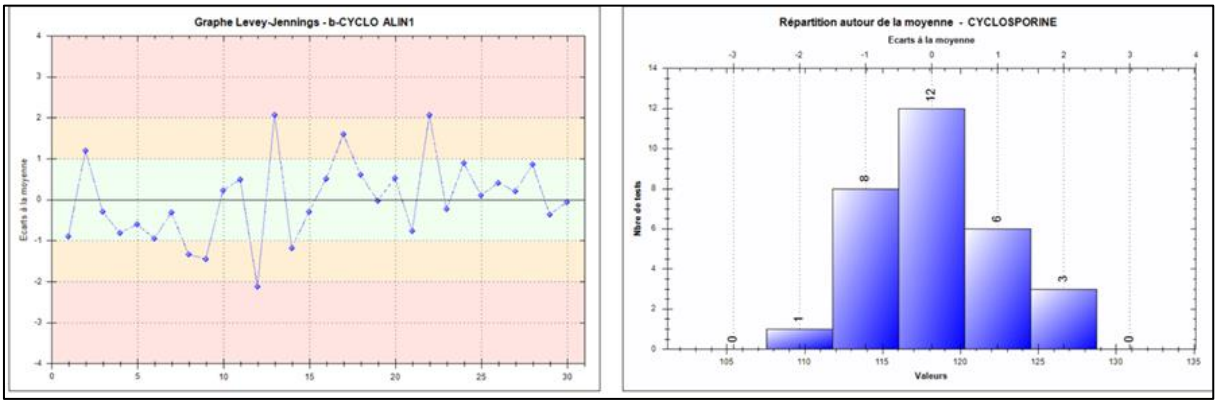

Figure 1 Low Level of Repeatability: Levey Jennings graph and the distribution around the mean

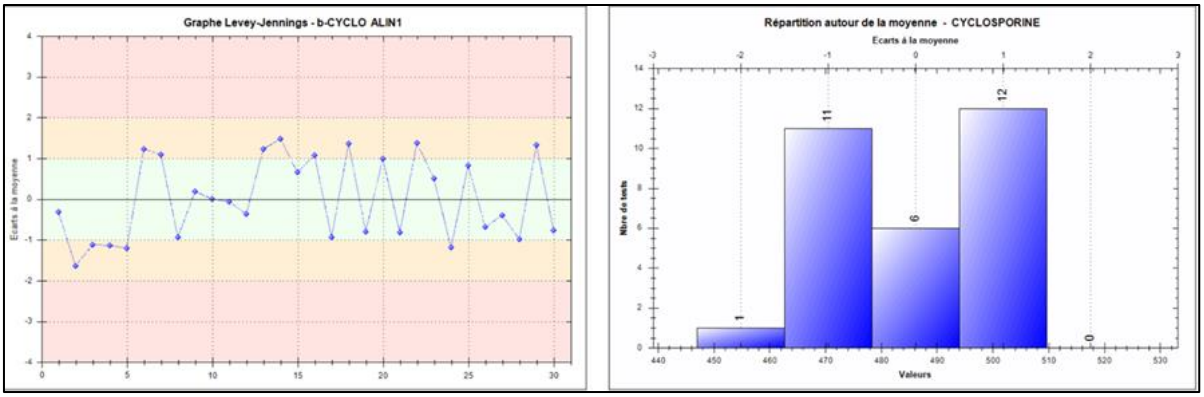

Figure 2 Medium Level of Repeatability: Levey Jennings graph and the distribution around the mean

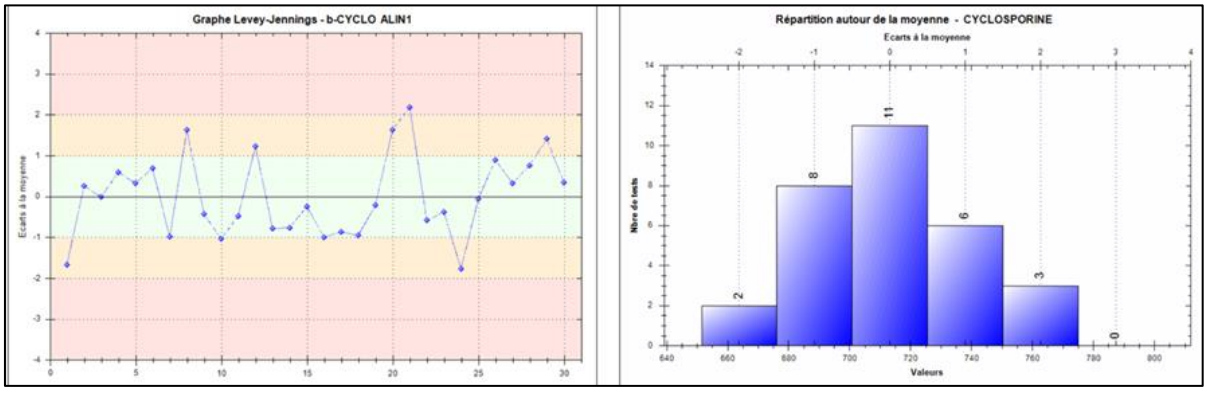

Figure 3 High Level of Repeatability: Levey Jennings graph and the distribution around the mean

3.2. Intermediate fidelity results

The outcomes of the intermediate fidelity examination yielded satisfactory results for all levels: low, medium, and high. Yielding coefficients of variation (CV1, CV2, and CV3) of 3.00%, 4.83%, and 6.01% respectively (Table 2). These findings have been graphically presented using Levey-Jennings plots (Fig. 4, Fig. 5, and Fig. 6) to further illustrate the obtained results.

Table 2 Reproducibility results for cyclosporine assay on the Alinity ci® automated system with comparison to the reference CV

Level of IQC	Numbers of value	Mean (ng/ml)	Standard deviation	Coefficient of variation CV (%)	Reference CV (%) set by the supplier
Low	30	108.21	3.251	3.00	5.4
Medium	30	404.98	19.563	4.83	5.4
High	30	694.49	41.729	6.01	8.5

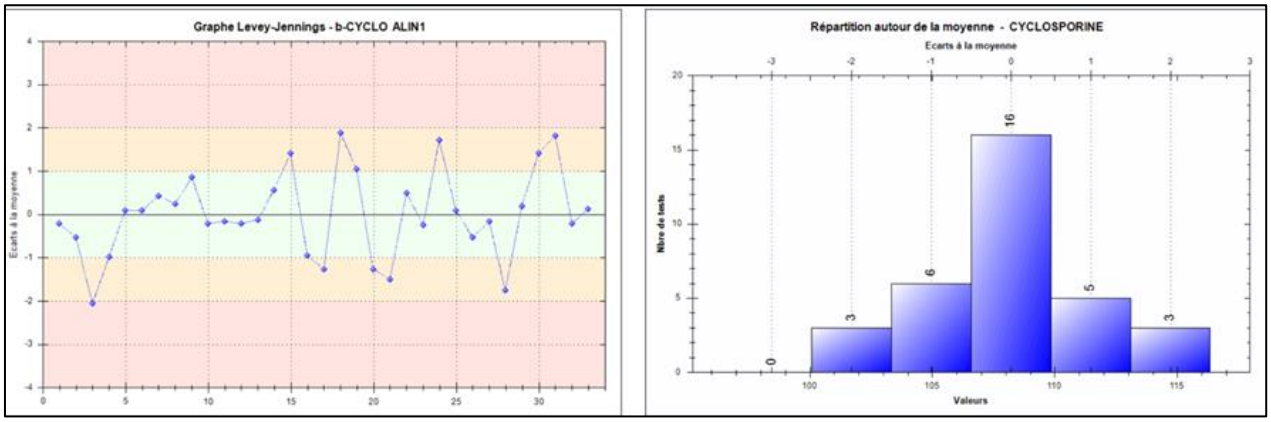

Figure 4 Low Level of Reproducibility: Levey Jennings graph and the distribution around the mean

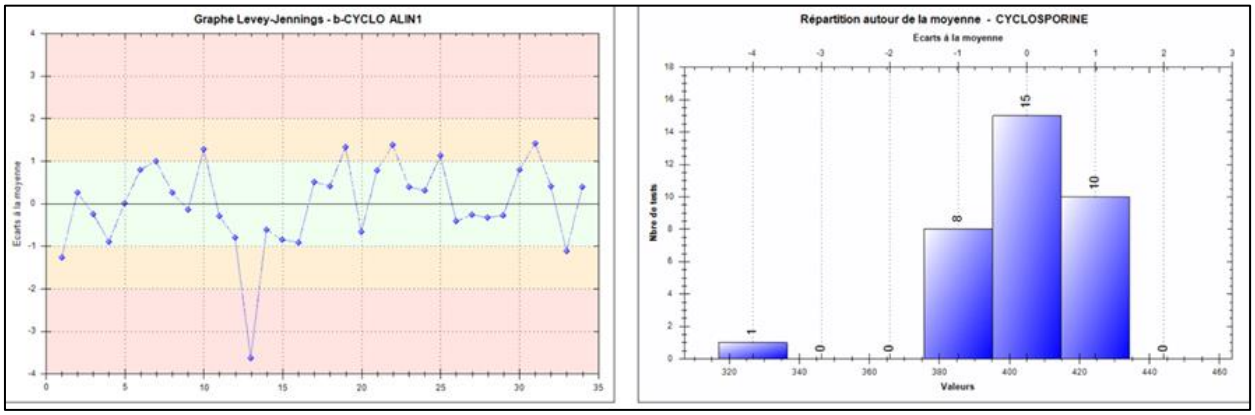

Figure 5 Low Level of Reproducibility: Levey Jennings graph and the distribution around the mean

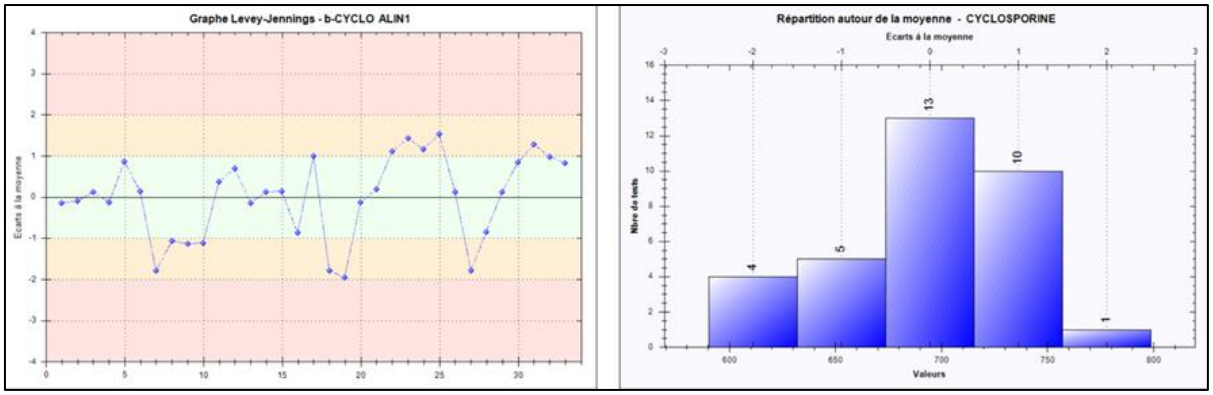

Figure 6 High Level of Reproducibility: Levey Jennings graph and the distribution around the mean

4. Discussion

The verification of an analytical method is an essential step in guaranteeing the quality and safety of results delivered to patients.(7) Repeatability and intermediate fidelity are statistical methods utilized in process control to assess the accuracy and consistency 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. Such measures are particularly valuable in decision support systems.(8)

The findings from the repeatability evaluation have confirmed that the immuno-chemiluminescent cyclosporine assay employed in our laboratory demonstrates remarkable precision. This is evidenced by consistently low coefficients of variation for repeatability, indicating minimal variability in repeated measurements conducted under identical conditions. These results not only underscore the reliability of this method but also highlight its stability and robustness.

The coefficients of variation and standard deviations obtained from the analysis of the intermediate fidelity were highly acceptable. Thus, fulfilling the supplier's stipulated requirements. These findings affirm that the immuno-chemiluminescent method used by the biochemistry laboratory of Mohammed VI university hospital for cyclosporine dosage on the Abbott Alinity ci® analyzer is not only consistent but also stable in replicating precise measurements across different concentration levels.

5. Conclusion

Given its narrow therapeutic index, cyclosporine dosing is essential for achieving high-quality patient care by balancing efficacy and adverse effects. Maintaining accuracy must remain the goal of each laboratory and would serve as their contribution to better patient outcomes. In line with this necessity, our study thoroughly tests the analytical performance of the immuno-chemiluminescent cyclosporine assay on the Abbott Alinity ci® analyzer. The results obtained from this verification affirm exceptional precision, consistency, and stability, meeting the supplier requirements.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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