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Verification of the analytical performance of the urine cannabinoids immunoassay on the Abbott Alinity ci ®

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Abstract

The precision and reliability of the urine cannabinoids assay on the Abbott Alinity ci® analyzer were systematically evaluated. The study was conducted over 30 days in the biochemistry laboratory of Mohammed VI University Hospital. Two phases were carried out: reproducibility assessment involving daily measurements across low and high cannabinoid levels, and repeatability testing involving 30 replicates per sample. A homogeneous immunoenzymatic approach was employed for cannabinoid quantification. Data analysis utilized the BYG middleware and adhered to the manufacturer standards.

For reproducibility, the coefficient of variation (CV) values were low, ranging from 2,6% to 3,1%. Repeatability CV values were also low, varying from 2,8% to 3,7%. Results were consistent with quality control limits, confirming the assay's precision and reliability.

This study highlights the robustness of the immunoenzymatic method on the Alinity ci® platform for urine cannabinoid analysis, with significant implications for accurate patient care. By adhering to stringent analytical performance verification protocols, laboratories ensure dependable and clinically meaningful outcomes. This research contributes to the foundational knowledge supporting the reliability of urine cannabinoid measurement methodologies.

Keywords: Urine cannabinoids assay; Abbott alinity ci analyzer; Precision; Reliability; Biochemistry laboratory

1. Introduction

The field of clinical diagnostics lies at the convergence of precision and innovation, where advancements in analytical methodologies meet the critical need for accurate patient evaluation. Among various clinical parameters, the detection and quantification of cannabinoids hold significant importance, emphasizing the necessity for precise measurements given the increasing prevalence of drug use. As diagnostic technologies evolve to meet growing demands, rigorous assessment of assay analytical performance becomes indispensable, with established methodologies such as homogeneous enzyme immunoassay providing reliable tools for accurate evaluation.

Delta-9-tetrahydrocannabinol (Δ9-THC) is widely recognized as the primary active compound in marijuana and/or hashish, responsible for its hallucinogenic and other physiological effects. Following inhalation or ingestion, Δ9-THC is rapidly absorbed and undergoes extensive metabolism. It is classified by the U.S. Drug Enforcement Administration (DEA) as Schedule I drugs, denoting compounds with no accepted medical applications and a high risk of abuse and dependency [1].

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The immunoassay utilizes monoclonal antibodies to detect the primary metabolite of Δ9-THC in urine. The Abbott Alinity ci® Analyzer represents a state-of-the-art advancement in clinical chemistry technology, designed to improve throughput, enhance operational efficiency, and ensure precise analytical performance. Verifying the analytical performance of assays on this platform is critical to maintaining reliable, data-driven diagnostic capabilities. Analytical method verification involves a systematic evaluation of a method's accuracy, precision, and reliability, performed according to standardized operational protocols [2].

This rigorous process provides laboratories with detailed insights into their analytical systems, including strengths, performance characteristics, and limitations. More importantly, it ensures that analytical results are dependable, enabling accurate clinical interpretations that support effective patient management and informed decision-making by healthcare providers.

2. Material and methods

This prospective study was conducted in the biochemistry laboratory of Mohammed VI University Hospital over a 30-day period. The investigation was divided into two distinct phases.

The first phase focused on evaluating the reproducibility of the analytical method by performing daily control measurements at three concentration levels—low and high—over the 30-day period to assess consistency.

In the second phase, a series of serum samples with cannabinoid concentrations evenly distributed across the measurement range were collected. These samples were categorized into three groups based on cannabinoid levels: low, medium, and high. For each sample, 30 replicates were conducted to evaluate repeatability.

Cannabinoid quantification was performed using the cannabinoid reagent kit on the chemistry module. Data processing was facilitated by the BYG middleware, which acts as an interface between the Alinity platform and the iLab result validation software. The coefficient of variation (CV) values obtained during the study were compared to reference standards established by reputable professional organizations, such as FSCB and RICOS.

3. Principle of the assay method

This assay utilizes monoclonal antibodies to detect the primary metabolite of Δ9-THC in urine. The technique relies on a competitive interaction between the enzyme-labeled analyte and the analyte present in the urine for binding to a fixed number of specific antibody sites.

When the analyte is absent from the sample, the specific antibody binds to the enzyme-labeled analyte, glucose-6-phosphate dehydrogenase (G6PDH), thereby inhibiting its enzymatic activity. This inhibition creates a direct correlation between the concentration of the analyte in the urine and the enzymatic activity.

G6PDH activity is quantified through spectrophotometric measurements at 340/412 nm (416 nm for c4000 and c16000 analyzers), based on its ability to convert nicotinamide adenine dinucleotide (NAD) to its reduced form, NADH.

4. Results

4.1. Reproducibility results

In the reproducibility test, the same sample is analyzed under various conditions to assess the impact of factors such as operators, time, reagent batches, and calibrations on the results. The objective is to establish acceptance criteria for prior analyses, particularly in decision support systems. The Coefficient of Variation (CV) is used to quantify the variability in the results.

For the low and high concentration levels, the following CV values were observed: CV1 = 2,6 %, CV2 = 3,1 %. These results are represented in the Levey-Jennings graphs (Fig. 1, Fig. 2).

The conclusion for each concentration level indicates that the CV for reproducibility is within acceptable limits, falling below the threshold for tolerance. Additionally, the limits set by the manufacturer, including their expansion factors, are referenced, and the observed CV values are compared to these standards (Table 1).

Table 1 Reproducibility results of urine assay by level with comparison to the manufacturer limits

Level of IQC	Number of values	Mean (ng/ml)	Standard Deviation	Coefficient of Variation CV (%)	Reference CV :
Low	30	69	1,7	2,6	3,6
High	30	60	1,5	3,1	3,6

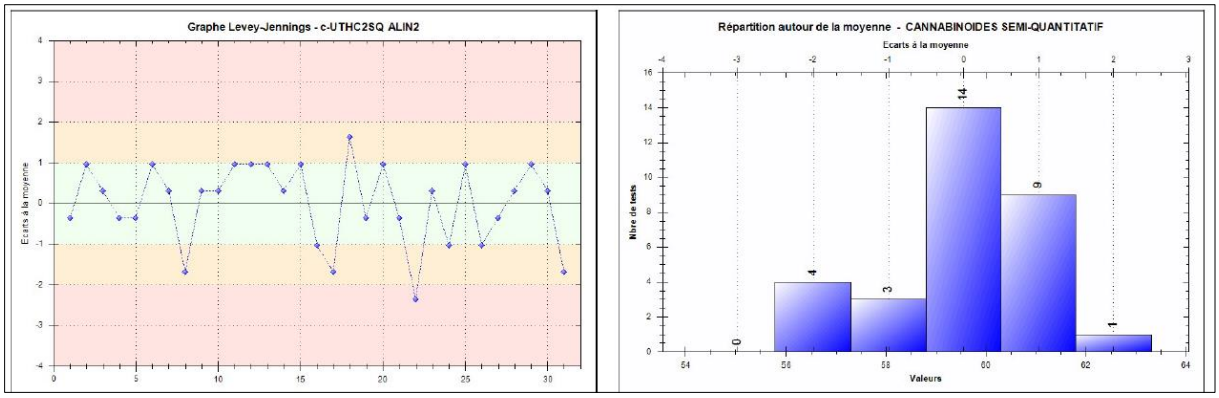

Figure 1 low Level of Reproducibility: Levey Jennings graph and the distribution around the mean – Cannabinoids

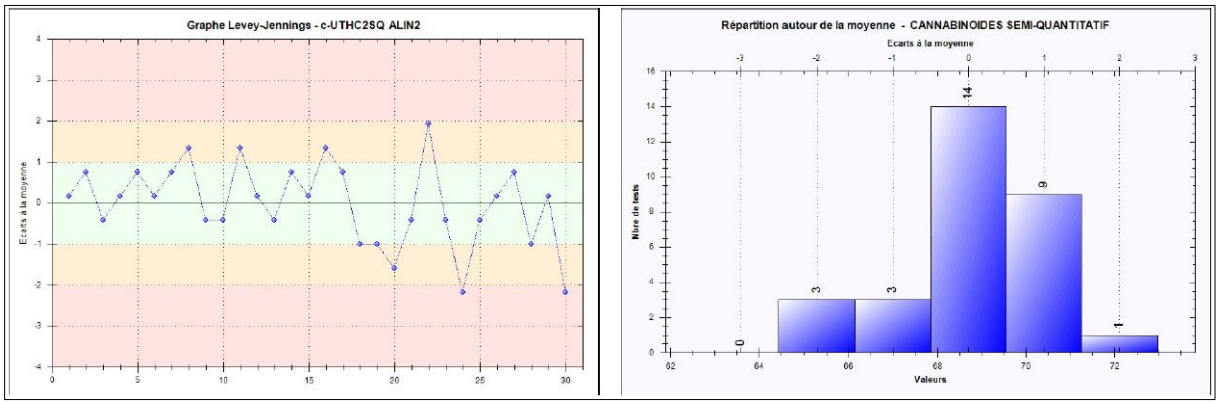

Figure 2 high Level of Reproducibility: Levey Jennings graph and the distribution around the mean – Cannabinoids

4.2. Repeatability Results

The repeatability test involves analyzing the same sample under optimal conditions to assess the performance and functionality of the system. The Coefficient of Variation (CV) is utilized to quantify variability in the results.

For the low, and high concentration levels, the observed CV values are as follows: CV1 = 3,7 %, CV2 =2,8 %. These results are depicted in the Levey-Jennings graphs (Fig. 3, Fig. 4).

The conclusion for each concentration level is that the CV for repeatability is accurate and remains below the accepted tolerance limits. As with the intermediate fidelity results, the manufacturer limits, including their expansion factors, are cited, and the CV values are compared to these established limits (Table 2).

Table 2 Repeatability results of urine assay by level with comparison to the manufacturer limits

Level of IQC	Number of values	Mean (ng/ml)	Standard Deviation	Coefficient of Variation CV (%)	Reference CV :
Low	30	60	1,4	3,7	4,6
High	30	69	1,4	2,8	3,8

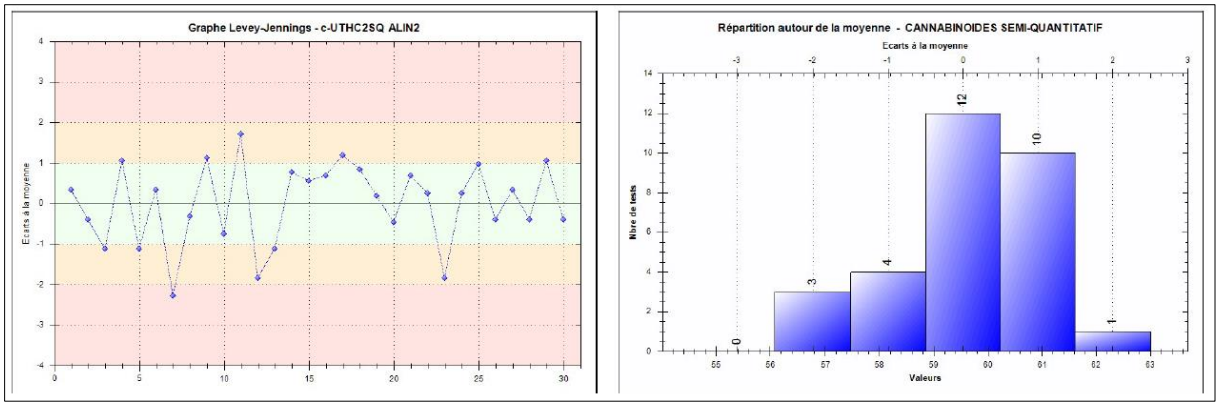

Figure 3 low Level of Repeatability: Levey Jennings graph and the distribution around the mean – Cannabinoids

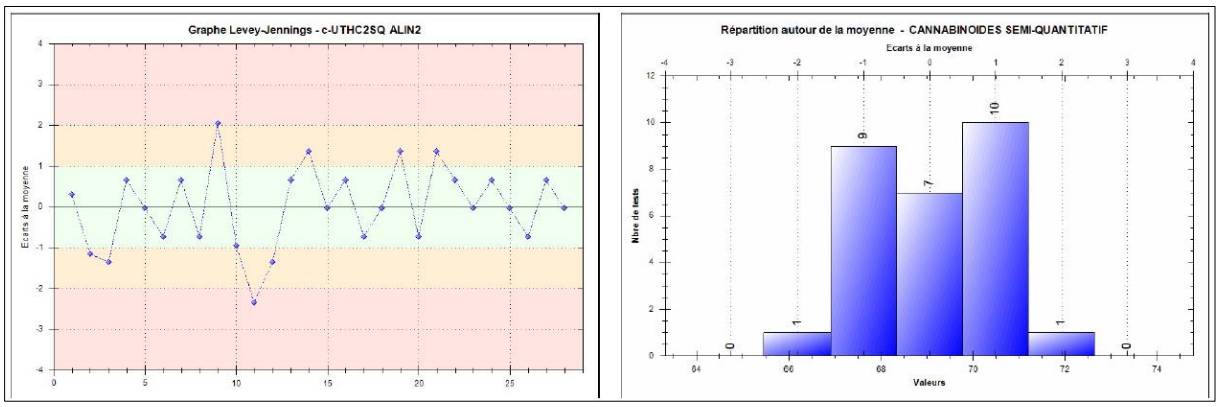

Figure 4 High Level of Repeatability: Levey Jennings graph and the distribution around the mean – Cannabinoids

In both cases, the conclusion is derived from comparing the observed CV values to predefined tolerance limits. The provided CV values reflect the degree of variability observed in the measurements. Since the calculated CV values fall below the established thresholds, it indicates that both the system's reproducibility and repeatability are within the acceptable range of performance.

5. Discussion

According to recent reports, cannabis remains the most widely used illicit drug [3,4], highlighting the growing necessity for accurate and reliable cannabinoids analysis. This underscores the critical need for robust testing methods to monitor usage, ensure regulatory compliance, and support clinical and forensic investigations.

The reproducibility test is a critical evaluation designed to assess the consistency of an assay's results under varying experimental conditions. It examines the influence of multiple factors, including differences in operators, time intervals, reagent lot variations, and calibration procedures, all of which may affect the reliability and robustness of the assay. To quantify the variability introduced by these factors, the Coefficient of Variation (CV) is utilized. The CV, expressed as a percentage, represents the ratio of the standard deviation to the mean, providing a normalized measure of data

dispersion. A lower CV indicates reduced variability and higher reproducibility, reflecting the assay's ability to yield consistent and reliable results across diverse operational conditions. This metric is essential for validating the precision and robustness of analytical methods in scientific and clinical settings.

For each of the low and high levels, the CV values are 2,6% and 3,1%, respectively. These values are relatively low, which implies that the assay is producing consistent results across different conditions.

The reproducibility findings demonstrate that the enzyme G6PDH competitive method exhibits robustness and stability under a range of experimental conditions. The low Coefficient of Variation (CV) values observed indicate minimal variability in assay results, even when critical factors such as operator technique or reagent lot are altered. This consistency ensures that the assay reliably generates results closely aligned with the mean value, a critical attribute for medical testing applications. Such reliability is essential in clinical diagnostics, where consistent and accurate results are imperative for informed decision-making. Furthermore, the alignment of the CV values with established quality control limits confirms that the assay adheres to industry standards for reproducibility, underscoring its suitability and reliability for precise diagnostic applications.

The repeatability test assesses the precision of the assay under tightly controlled and optimal conditions, evaluating its ability to produce consistent results when the same sample is analyzed in multiple replicates. The Coefficient of Variation (CV) values for repeatability were determined to be exceptionally low, with CV1 = 3,7%, CV2 = 2,8%. These values indicate minimal variability, underscoring the assay's high level of precision.

The findings from the repeatability analysis demonstrate that the enzyme G6PDH method yields highly consistent and accurate measurements when the same sample is tested repeatedly. The low CV values further emphasize the assay's stability and reliability under standardized conditions.

This degree of precision is particularly critical in clinical diagnostics, where even slight variations in results can have significant implications for patient management. Additionally, the close alignment of the CV values with established quality control benchmarks confirms the assay's compliance with industry standards, solidifying its suitability for applications requiring reproducible and dependable results in clinical and diagnostic settings.

The reproducibility and repeatability results collectively confirm the robustness and reliability of the serum glucose assay based on the immuno-enzymatic method. The assay demonstrates minimal variability and high precision across diverse conditions and repeated measurements of the same sample.

These attributes are fundamental in in impairment assessments, drug abuse monitoring, and legal investigations, where accurate and consistent results are essential for patient care. furthermore, the comparison with quality control standards offers an objective validation of the assay’s performance, reinforcing its reliability for researchers and healthcare professionals. The comprehensive evaluation of variability ensures adherence to industry standards, underscoring the assay’s suitability for clinical decision-making.

6. Conclusion

This study demonstrated the robust analytical performance of the cannabinoid immunoassay on the Abbott Alinity ci® platform, confirming its high reproducibility and repeatability. The low coefficient of variation (CV) values observed indicate minimal variability and strong assay reliability under different operational conditions. These findings validate the precision and stability of the method, ensuring accurate detection and quantification of cannabinoids in urine. By adhering to established quality control standards, this assay provides a dependable tool for clinical diagnostics, forensic investigations, and substance abuse monitoring. Ultimately, this research contributes to improving public health outcomes by enhancing the accuracy of drug testing methodologies and reinforcing evidence-based decision-making.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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