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

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Abstract

The precision and reliability of the urine cannabinoids immunoassay on the Abbott Alinity ci® analyzer were systematically evaluated over 30 days in the biochemistry laboratory of Mohammed VI University Hospital. The study consisted of two phases: reproducibility assessment, involving daily measurements at low and high cannabinoid concentrations, and repeatability testing, with 30 replicates per sample. Cannabinoid quantification was performed using a homogeneous immunoenzymatic assay, with data analysis conducted via the BYG middleware, following the manufacturer's guidelines.

The coefficient of variation (CV) for reproducibility ranged from 2.6% to 3.1%, while repeatability CV values varied between 2.8% and 3.7%, all within quality control limits, confirming the assay's high precision and reliability. These findings underscore the robustness of the Alinity ci® platform for urine cannabinoid analysis, ensuring accurate and reproducible results critical for clinical and forensic applications. By adhering to rigorous analytical performance verification protocols, laboratories can ensure dependable and clinically meaningful outcomes, reinforcing the reliability of urine cannabinoid testing methodologies.

Keywords: Urine Ecstasy Assay; Abbott Alinity Ci Analyzer; Precision; Reliability; Biochemistry Laboratory

1. Introduction

The field of clinical diagnostics is positioned at the intersection of precision and innovation, where advancements in analytical methodologies align with the imperative for accurate patient evaluation. Among various clinical parameters, the detection and quantification of ecstasy hold significant importance, underscoring the necessity of precise measurements given the rising prevalence of drug abuse. As diagnostic technologies advance to address increasing demands, rigorous evaluation of assay analytical performance becomes essential, with proven methodologies such as homogeneous enzyme immunoassay serving as reliable tools for accurate assessment.

Ecstasy, refers to a group of amphetamine analogs substituted on the ring structure by a methylenedioxy group, including 3,4-methylenedioxyamphetamine (MDA), 3,4 methylene dioxy methamphetamine (MDMA), and 3,4-methylenedioxyethylamphetamine (MDEA). These are central nervous system stimulants whose abuse is widespread due to their psychotropic effects. They have been classified by the U.S. Drug Enforcement Administration (DEA) under Schedule I, indicating substances with no recognized medical use and a high potential for addiction [1].

The Abbott Alinity ci® Analyzer exemplifies advanced technological innovation in clinical chemistry instrumentation, designed to enhance throughput, operational efficiency, and analytical precision. Ensuring robust, data-driven

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diagnostic capabilities necessitates a thorough verification of the analytical performance of assays conducted on this platform. Analytical method verification is a systematic process designed to evaluate the capabilities and reliability of an analytical method. This procedure entails quantification performed in accordance with a standardized operational protocol to ensure accuracy, precision, and consistency in results [2]. This methodological evaluation provides laboratories with detailed insights into their analytical techniques, performance parameters, and limitations. Crucially, it ensures that these performance metrics uphold the reliability of analytical results, enabling clinically meaningful interpretations that benefit both patients and healthcare providers.

2. Material and methods

This prospective study was carried out in the biochemistry laboratory of Mohammed VI University Hospital over a 30-day period. The study was divided into two distinct phases. The first phase focused on evaluating the reproducibility of the analytical method by performing daily control measurements at two distinct concentration levels—low and high—over the 30-day period to assess consistency.

In the second phase, a series of serum samples with ecstasy concentrations evenly distributed across the measurement range were collected. These samples were categorized into two groups based on ecstasy levels: low and high. For each sample, 30 replicates were performed to determine repeatability. The ecstasy measurement was conducted using the ecstasy reagent. Data processing was facilitated by the BYG middleware, which serves as an interface between the Alinity platform and the iLab result validation software. The coefficient of variation (CV) values obtained from the study were compared to the reference standards established by reputable professional organizations, including the FSCB and RICOS.

2.1. Principle of the assay method

This assay employs monoclonal antibodies designed to detect ecstasy in urine with minimal cross-reactivity to other amphetamine derivatives. The assay operates on the principle of competition between the enzyme-labeled analyte and the analyte present in the urine, both competing for binding sites on a specific antibody. In the absence of analyte in the sample, the specific antibody binds to the enzyme-labeled analyte, glucose-6-phosphate dehydrogenase (G6PDH), thereby inhibiting enzymatic activity. This creates a direct correlation between the concentration of the analyte in the urine and the enzyme activity. The G6PDH enzymatic activity is measured spectrophotometrically at 340/412 nm (416 nm for c4000 and c16000 analyzers), based on its ability to convert nicotinamide adenine dinucleotide (NAD) to NADH. This test provides a sensitive, specific, and rapid means to detect ecstasy in urine, with minimal interference from other substances in the amphetamine class.

3. Results

3.1. Reproducibility results

In the reproducibility test, the same sample is analyzed under varying conditions to assess how factors like operators, time, reagent batches, and calibrations affect the results. The goal is to establish acceptance criteria for priors, especially in decision support systems. The Coefficient of Variation (CV) is used to measure the variability of the results.

-For the low and high levels, the CV values are provided (CV1 = 1,3 %, CV2 = 1,8%) These results are illustrated on the Levey-Jennings graphs (Fig. 1, fig. 2).

The argument of the conclusion for each level is that the CV of Reproducibility is correct and falls below the tolerated limit. (Table. 1).

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

Level of IQC	Number of values	Mean (ng/ml)	Standard Deviation	Coefficient of Variation CV (%)	Reference CV : (%)
Low	30	655	4.9	1.3	2
High	30	671	4.9	1.8	2.1

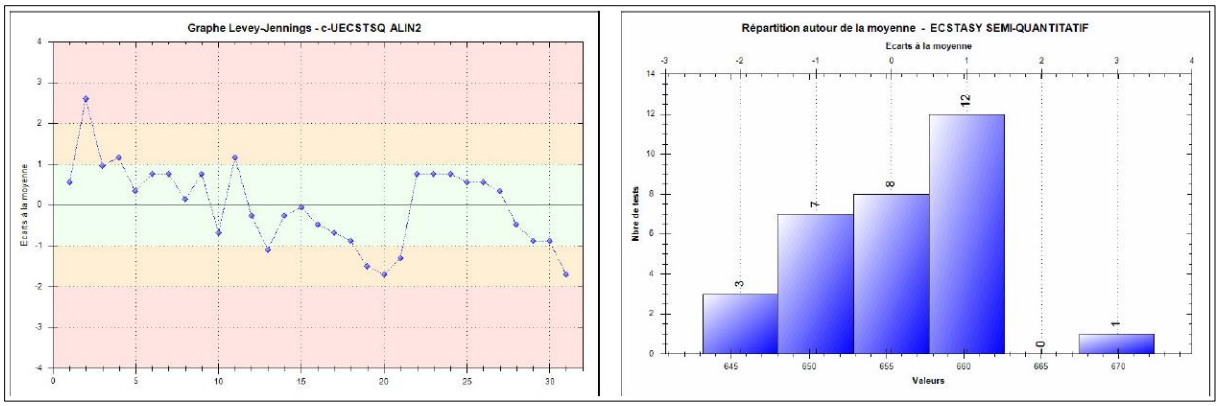

Figure 1 low Level of Reproducibility: Levey Jennings graph and the distribution around the mean – ecstasy

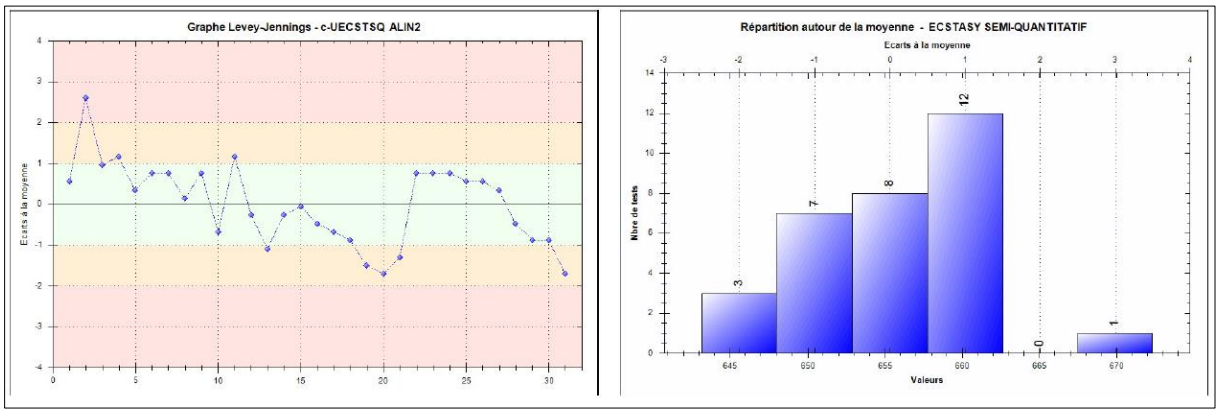

Figure 2 high Level of Reproducibility: Levey Jennings graph and the distribution around the mean – ecstasy

3.2. Repeatability Results

The repeatability test involves analyzing the same sample under optimal conditions to assess the system's performance and functionality. CV values are again used to measure variability.

For the low and high levels, the CV values provided are 1% for both levels. These results are illustrated on the Levey-Jennings graphs (Fig. 3, fig. 4)

The argument of the conclusion for each level is that the CV of Repeatability is correct and falls around the tolerated limit. (Table. 2).

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

Level of IQC	Number of values	Mean (ng/ml)	Standard Deviation	Coefficient of Variation CV (%)	Reference CV : (%)
Low	30	659	4.5	1	1
High	30	663	5.1	1	0.8

In both cases, the conclusion is drawn by comparing the coefficient of variation (CV) values to predefined tolerance limits. The reported CV values quantify the variability observed in the measurements. The fact that the calculated CV values fall below and around the specified limits indicates that the system demonstrates acceptable reproducibility and repeatability, ensuring its reliability for analytical applications.

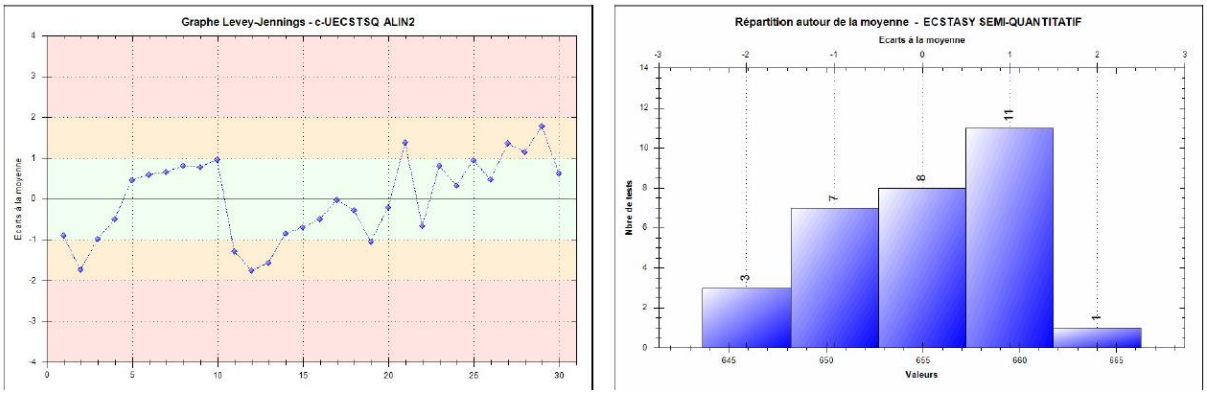

Figure 3 low Level of Repeatability: Levey Jennings graph and the distribution around the mean – ecstasy

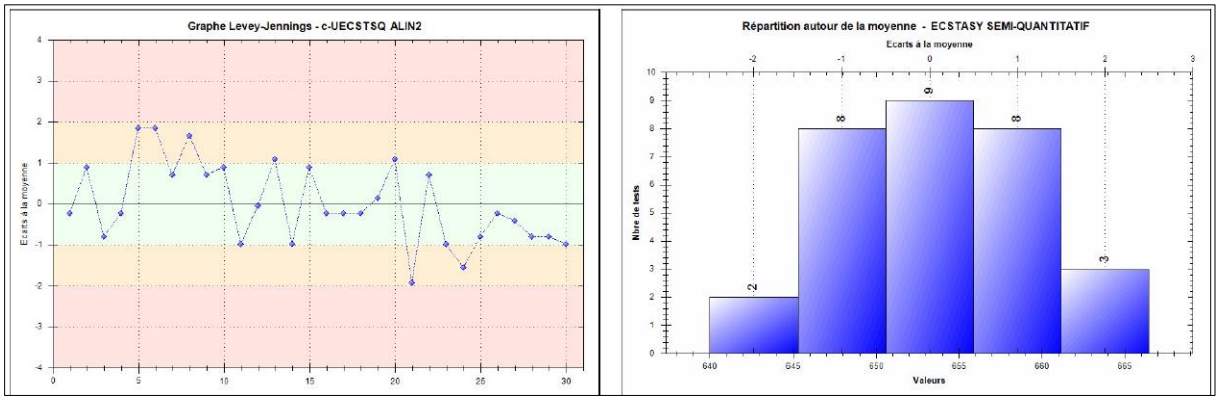

Figure 4 High Level of Repeatability: Levey Jennings graph and the distribution around the mean – ecstasy

4. Discussion

The immunoassay-based detection of ecstasy (MDMA) in urine is widely used in toxicology and forensic analysis due to its rapid screening capabilities. However, ensuring the analytical performance of such assays is crucial for obtaining accurate and reliable results in clinical and regulatory settings. The Abbott Alinity ci® system employs an immunoassay designed for the qualitative detection of MDMA metabolites, yet its sensitivity, specificity, and precision require thorough validation. This study evaluates the assay's reproducibility, repeatability, and accuracy, contributing to the optimization of MDMA screening protocols in routine testing [3,4].

The reproducibility assessment is a fundamental evaluation aimed at determining an assay's consistency under varying experimental conditions. This analysis accounts for multiple influencing factors, including operator variability, time-dependent fluctuations, reagent lot differences, and calibration procedures, all of which may impact the assay's reliability and robustness. To quantitatively assess the variability introduced by these factors, the Coefficient of Variation (CV**)** is employed. Expressed as a percentage, the CV represents the ratio of the standard deviation to the mean, serving as a standardized measure of data dispersion. A lower CV signifies minimal variability and enhanced reproducibility, demonstrating the assay's capability to generate consistent and reliable results across different operational conditions. This parameter is crucial for verifying the precision and robustness of analytical methodologies in both scientific research and clinical diagnostics.

For each of the low and high levels, the CV values are 1,3% and 1,8%, respectively. These values are relatively low, indicating that the assay maintains a high level of consistency across varying conditions.

The reproducibility assessment confirms that the immuno-enzymatic method demonstrates strong robustness and stability across diverse experimental conditions. The consistently low Coefficient of Variation (CV) values indicate minimal variability in assay performance, even when key factors such as operator technique or reagent lot changes. This high level of consistency ensures that the assay reliably produces results closely aligned with the expected mean, a critical requirement for medical testing. Such reliability is particularly essential in clinical diagnostics, where precise

and reproducible outcomes are crucial for informed decision-making. Moreover, the alignment of the CV values with established **quality control** thresholds validates the assay's compliance with industry standards, reinforcing its suitability for accurate and reliable diagnostic applications.

The repeatability assessment evaluates the assay's precision under strictly controlled and optimal conditions, measuring its ability to generate consistent results across multiple replicates of the same sample. The Coefficient of Variation (CV) values for repeatability were found to be slightly above the limits (1% for both levels) indicating minimal variability and reinforcing the assay's high degree of precision.

The results of this analysis confirm that the immuno-enzymatic method produces highly consistent and accurate measurements when the same sample is repeatedly analyzed. The low CV values further highlight the assay's stability and reliability under standardized conditions. The analysis of ecstasy is essential for monitoring intoxications, preventing overdoses, and controlling illicit drug trafficking. Additionally, it plays a crucial role in epidemiological studies, aiding in the assessment of consumption trends and the development of harm reduction strategies.

This level of precision is particularly crucial in clinical diagnostics, where even minor variations can't be tolerated. Therefore, the close alignment of the CV values with established quality control benchmarks validates the assay's compliance with industry standards, demonstrating its suitability for applications requiring reproducible and dependable results in clinical and diagnostic settings.

5. Conclusion

This study confirms that the immuno-enzymatic assay on the Abbott Alinity ci® platform demonstrates excellent reproducibility and repeatability, with low Coefficient of Variation (CV) values indicating high precision and reliability in detecting ecstasy in urine. The consistency of these results with established quality control standards validates the assay's analytical performance, ensuring accurate and dependable measurements under varying conditions. These findings highlight its applicability in clinical and forensic toxicology, supporting drug screening, overdose prevention, and regulatory compliance. By enhancing the accuracy of ecstasy detection, this study contributes to public health initiatives and underscores the importance of continuous advancements in diagnostic technologies.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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