



Investigating False Positive Results in Urine Analysis Using the Immunoassay Method for Substance Metabolite Detection: A Retrospective Analysis

Doğrulama İstenen İdrarda Madde Analiz Sonuçlarının İncelenmesi

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Abstract

Aim This study aims to investigate the false positive outcomes in urine analysis via the immunoassay method within an addiction treatment center outpatient clinic. While widely utilized for substance detection, false positive results in the immunoassay method can cause misleading. This study aims to attract attention to false positivity and its implications.

Material and Method Conducted at an Alcohol and Drug Addiction Center outpatient clinic, the study retrospectively examines urine analyses from February to May 2023. Among 5109 immunoassay-based urine analyses, only 25 were subjected to confirmation through liquid chromatography-tandem mass spectrometry (LC-MS).

Results The findings demonstrate that LC-MS verified 40% of immunoassay-positive urine samples as true positives. Significantly, false positive results were notable, particularly in cases involving benzodiazepines. The study accentuates a noteworthy disparity between initial immunoassay outcomes and subsequent confirmatory tests, casting doubts on the reliability of the immunoassay method. A fundamental discovery is the consistent identification of pregabalin and gabapentin in urine samples yielding false positive benzodiazepine results during confirmation analysis. This revelation prompts inquiries into the potential cross-reactivity of these medications in immunoassay-based tests, suggesting the need for careful consideration in clinical and forensic contexts.

Conclusion The study underscores the importance of confirmatory testing for result accuracy and the multifaceted implications of false positives on patient-doctor relationships, treatment decisions, and patient safety. Acknowledging the study's limitations, such as its retrospective nature and limited participant pool, the research underscores the requirement for a comprehensive approach to substance detection, merging screening and confirmatory analyses to enhance diagnostic dependability.

Keywords Benzodiazepine, false positive, immunoassay, LC-MS, opioid.

Özet

Amaç Bağımlılık yapan maddelerin taranması ve tespiti, gerek kişinin tedavi süreci, gerekse adli boyutları için önem arz etmektedir. Sıklıkla kullanılan bir yöntem olan idrarda immunoassay yöntemi ile madde tespiti, zaman zaman yanlış-pozitif sonuçlar verebilmektedir. Bu çalışmada, kliniğimizde immunoassay ile idrarda madde metaboliti saptanan sonuçların ne kadarının yanlış-pozitif olduğunun incelenmesi amaçlanmıştır.

Gereç ve Yöntem Bir AMATEM kliniğinde Şubat ve Mayıs 2023 tarihleri arasında yapılan idrar analizlerinin retrospektif olarak incelenmesi ile bu çalışma gerçekleştirilmiştir. 5109 hastanın verisi taranmış ve çalışma için uygun olan 25 hasta çalışmaya dahil edilmiştir; kişilerin yaş, cinsiyet, medeni durum ve idrar analiz sonuçlarına hastane kayıtlarından ulaşılmış, kendileriyle yüz yüze görüşülmüştür. Çalışma için etik onam alınmıştır (no: E-23/1319).

Bulgular Çalışmanın sonuçlarına göre, LC-MS ile immunoassay yöntemiyle pozitif sonuç veren idrar örneklerinin sadece %40'ı pozitif olarak saptanmıştır. Opioid pozitifliğiyle doğrulamaya gönderilen idrarların sadece 3 (%23,1) tanesinin, benzodiazepin pozitifliğiyle doğrulamaya gönderilen idrarların ise 7 (%58,3) tanesinin pozitifliği konfirme edilmiştir. Benzodiazepin doğrulama sonucunda pozitif gelenlerin tamamında pregabalin ve gabapentin tespit edilmesi de çalışmamızın önemli bulgularından bir tanesidir.

Sonuç Çalışmamızın bulguları incelendiğinde, 5109 idrar analizinden sadece 25 tane doğrulama istenmiş olması ve sadece opioid ile benzodiazepin pozitifliğinde doğrulama istenmiş olması dikkate değerdir. İdrarda herhangi bir maddenin pozitifliği saptandığında, hastalar sık sık bunun yanlış pozitif olabileceğini iddia etmektedirler ve çalışmamızın sonuçlarına göre doğrulamaya gönderilen idrarların %60'ının yanlış-pozitif olduğu saptanmıştır. Son yıllarda kötüye kullanımı katanarak artan pregabalin ve gabapentinin benzodiazepin yanlış-pozitifliğine neden olabileceğini ve immunoassay analizlerinin yanlış-pozitif sonuçlanabileceğini, bağımlılık alanında çalışan psikiyatristlerin göz önünde bulundurmalarında fayda vardır.

Anahtar Kelimeler Benzodiazepin, immunoassay, LC-MS, opioid, yanlış pozitif.

INTRODUCTION

Detecting addictive substances in biological samples, particularly urine plays a significant role in clinical and legal contexts.¹ The immunoassay method has gained prominence as a reliable screening tool for detecting abused substances in urine samples due to its simplicity and availability.^{2,3} However, despite its widespread use, the immunoassay method is not devoid of limitations, occasionally leading to false positive results.⁴ The antibodies used in the immunoassay method can cause cross-reactivity and false-positivity.⁵ Incorrect positive urine analysis results during the treatment processes of patients with substance use disorder will hinder the proper execution of this process.^{6,7}

In the mass spectrometry technique, the relevant substance is directly detected, unlike the indirect measurement in the immunoassay method. Therefore, it is considered the best analytical technique for the most accurate substance screening analyses.⁸ However, it is not widely used due to the lack of mass spectrometry equipment in every laboratory or the delay in obtaining analysis results for days. There are types, such as liquid chromatography-tandem mass spectrometry (LC-MS) or gas chromatography-tandem mass spectrometry (GC-MS).⁹

This study aims to explore the false positive results in urine analysis with the immunoassay method in an addiction treatment center outpatient. By conducting retrospective research, we intend to shed light on the prevalence, factors, and implications of false positive results in this diagnostic approach.

MATERIALS and METHODS

The study was conducted at the Alcohol and Drug Addiction Center outpatient clinics of Ankara Training and Research Hospital. Samples found positive for substance metabolites in urine analyses performed by the immunoassay method were analyzed by the LC-MS method for confirmation.

Records from February to May 2023 were retrospectively reviewed, revealing 5109 urine analyses conducted using the immunoassay method. Among these, it was observed that only 25 were sent for confirmation through LC-MS analysis. These 25 patients who had been referred for confirmation due to positive immunoassay-based urine analysis results were included in the study.

By reviewing the hospital's medical records, essential demographic information, including age, gender, marital status, and initial urine analysis results, were extracted. The study design did not entail direct interaction with the participants; the analysis was solely based on the available data. Ethical approval for the study was obtained from the Ankara Training and Research Hospital Clinical Studies Ethics Committee (decision no: E-23/1319).

The research data were analyzed using SPSS (Statistical Package for the Social Sciences for Windows v.22.0, SPSS Inc., Chicago, IL). Descriptive statistics were presented as mean (\pm) standard deviation, frequency distribution, and percentage.

RESULTS

The participants exhibited a gender distribution, with 88% male and 12% female. The demographic landscape encompassed various marital statuses, including 56% single, 20% married, and 24% divorced or widowed individuals. The average age of the participants was calculated to be 35.6 ± 9.8 years.

Of the 25 urine samples, 13 were sent for confirmation due to opioid and 12 benzodiazepine positivity. Upon confirmation analysis, 40% of the urine samples were validated as positive, whereas the remaining 60% were negative (Table 1).

Table 1. Socio-demographic Characteristics of Participants

	mean / n	SD / %
Age	35.6	9.8
Gender		
Male	22	88
Female	3	12
Marital status		
Single	14	56
Married	5	20
Divorced/widowed	6	24
Reason for confirmation		
Opioid+	13	52
Benzodiazepine+	12	48
Confirmation result		
Positive	10	40
Negative	15	60

Mean: mean; n: number; SD: standard deviation; %: percentage.

Moreover, a closer examination of the samples revealed that only 23.1% of the urine samples sent for confirmation due to opioid positivity were eventually confirmed as positive, highlighting a noteworthy discordance between initial immunoassay results and subsequent confirmatory tests. Similarly, among the samples sent for benzodiazepine confirmation, a significantly higher proportion (58.3%) were confirmed as positive, indicating that many benzodiazepine-positive results from the immunoassay might be false positives.

One of the crucial results of this study was the consistent identification of pregabalin and gabapentin in all cases that yielded positive results for benzodiazepines during the confirmation analysis. This observation raises intriguing questions about the potential cross-reactivity of these medications in immunoassay-based tests, suggesting a nuanced consideration of their presence in clinical and forensic contexts (Table 2).

Table 2. Analysis of Groups Sent for Confirmation Due to Opioid or Benzodiazepine Positivity

	Opioid+ (n=13)	Benzodiazepine+ (n=12)
Confirmation Result		
Positive	3 (23.1%)	7 (58.3%)
Negative	10 (76.9%)	5 (41.7%)
Substances Detected upon Confirmation		
Pregabalin	0	5
Gabapentin	0	2
Morphine	3	0

n: number; %: percentage.

DISCUSSION

According to the findings of our study, only 25 confirmatory tests were sent out of 5109 urine analyses. Notably, more than half of the positive samples for the substance metabolites were found negative, according to the confirmation results. Gabapentin and pregabalin were found to cause benzodiazepine false positivity in the immunoassay method.

The implications of false positive results in urine analysis using the immunoassay method are multifaceted. A false positive result in urine analysis can have negative consequences for patients. Patients under treatment may be mistakenly categorized as having used heroin when they have not, which can undermine trust in the patient-doctor relationship.⁹

Another example is when someone who has used medication containing codeine for a cough is mistakenly categorized as having an opioid overdose due to a false positive urine result when an underlying issue affects their clinical condition. This misclassification might lead to overlooking the actual underlying problem.¹⁰

False positive benzodiazepine results can disrupt the treatment process for patients under buprenorphine therapy, as the co-administration of benzodiazepines and opioids can lead to respiratory depression.¹¹ Due to this potential risk,

clinicians might refrain from prescribing buprenorphine treatment to such patients.¹²

The present study not only emphasizes the need for confirmatory testing to ensure accurate results but also emphasizes the significance of understanding the factors that can contribute to false positive outcomes. The prevalence of false positive results in the studied cases, particularly in benzodiazepine-related instances, serves as a reminder that caution is required in interpreting immunoassay-based findings, especially in the context of substances that might share structural similarities.

As the abuse of substances like pregabalin and gabapentin gains momentum, their potential to trigger false positive benzodiazepine results warrants thorough consideration. Professionals in addiction psychiatry should be vigilant about the limitations and potential pitfalls of immunoassay methods, acknowledging the need for a comprehensive approach to substance detection that combines screening and confirmatory analyses.¹³

Our study also has certain limitations that need to be acknowledged. Firstly, its retrospective design and the derivation of data from health records restrict the generalizability of the results. Additionally, the limited number of participants constitutes another shortcoming of our study. Besides these limitations, this retrospective analysis sheds light on the intricate dynamics of false positive results in urine analysis using the immunoassay method. By providing insights into these results' prevalence, patterns, and implications, the study underlines the necessity of a holistic approach to substance detection, ensuring the reliability of diagnostic outcomes and the accuracy of conclusions drawn.

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The authors declare they have no conflicts of interest to disclose.

Ethical Approval

University of Health Science, Ankara Education and Research Hospital ethics Committee and following the Declaration of Helsinki (decision no: E-23/1319).

Peer-review

Externally and internally peer-reviewed.

Authorship Contributions

Concept: G.Z.İ., Ş.B.N., Design: G.Z.İ., Ş.B.N., Data collection or Processing: G.Z.İ., Analysis or interpretation: G.Z.İ., Ş.B.N., Literature Search: G.Z.İ., Ş.B.N., Writing: G.Z.İ., Ş.B.N.

Conflict of Interest

The authors declare that they have no conflict of interest.

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