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Araştırma Makalesi / *Research Article*

Comparison of Urine Drug Abuse Testing Analyzed in Glass and Plastic Tubes

Cam ve Plastik Tüplerde İdrarda Uyuşturucu Bağımlılığı Testlerinin Karşılaştırılması

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ABSTRACT

Objectives: Drug abuse tests are applied to determine drug use to combat disease, crime, or substance abuse. We aimed to investigate whether cheaper and more practical plastic tubes can be an alternative to glass tubes for drug abuse analysis in urine.

Material and Methods: Paired fresh urine samples from 80 volunteers were collected into glass and plastic tubes, and drug abuse tests were analyzed in parallel after specimen validity tests. Amphetamines (AMP), benzodiazepines (BNZ), cocaine (COC), opiates (OPI), and cannabinoids (THC) were measured semi-quantitatively using the immunoassay method with the Roche Cobas c 501. Cut-off values for positivity were taken as AMP > 500 μg/L, BNZ > 300 μg/L, COC > 150 μg/L, OPI > 2000 μg/L, and THC > 50 μg/L. To investigate the effect of time, 32 of the samples were stored at room temperature for 4 hours without any preservative and reanalyzed. The SPSS 25.0 program was used to analyze the data.

Results: There were no significant differences between glass and plastic tubes in AMP, BNZ, COC, OPI, and THC concentrations. A statistically significant difference was found for OPI ($p = 0.005$), but the difference in OPI levels did not change the clinical decision. There were high correlations between all results for the same analyte measurements in both tubes ($p = 0.0001$). Additionally, 4-hour storage at room temperature did not cause degradation or adsorption of any drug.

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Conclusion: As a result of this study, it can be concluded that plastic tubes can be used instead of glass tubes in the analysis of drugs of abuse, with no significant change in substance concentrations after storage for four hours at room temperature.

Key Words: Urine, Drug abuse testing, Substance abuse

ÖZET

Amaç: Madde bağımlılığı testleri tıbbi, adli vakalarda ve denetimli serbestlik hastalarında uyuşturucu kullanımının tespiti amacıyla kullanılmaktadır. Biz bu çalışmada idrarda madde analizinde ucuz ve pratik olan plastik tüplerin cam tüplere alternatif olup olamayacağını araştırmayı amaçladık.

Gereç ve Yöntem: 80 yetişkin gönüllüden alınan taze idrar örnekleri cam ve plastik tüplere aktarıldı ve örneklerde idrar bütünlük testleri çalışılarak örneklerin uygunlukları değerlendirildi. Ardından idrarda amfetaminler (AMP), benzodiazepinler (BNZ), kokain (COC), opiatlar (OPI) ve kannabinoidlerin (THC) düzeyi Roche Cobas c 501'de immünoanaliz yöntemiyle yarı kantitatif olarak ölçüldü. Testin pozitifliği için kesim değerleri; AMP> 500μg/L, BNZ> 300μg/L, COC> 150μg/L, OPI> 2000μg/L ve THC> 50μg/L olarak alındı. İdrar örneklerinin bekletilmesinin test sonucuna etkisini araştırmak için, numunelerden 32 tanesi herhangi bir koruyucu madde olmadan dört saat bekletildi ve tekrar analiz edildi. Verilerin analizinde SPSS 25.0 programı kullanıldı.

Bulgular: AMP, BNZ, COC, OPI ve THC konsantrasyonlarında cam ve plastik tüpler arasında anlamlı bir fark yoktu. OPI açısından istatistiksel olarak anlamlı fark bulundu (p= 0.005) ancak OPI düzeylerindeki değişiklik klinik kararı (pozitiflik) değiştirmedi. Her iki tüpte de aynı analit ölçümüne ilişkin tüm sonuçlar arasında anlamlı yüksek korelasyon vardı (p= 0.0001). Bekletilen idrar numunelerinde (n= 32) sadece BNZ plastik tüpte istatistiksel olarak anlamlı yüksek bulundu (p= 0.037); ancak bu farklılıklar örneklerin pozitifliğini veya negatifliğini değiştirmedi ve klinik olarak anlamsızdı. Tüm testler için tüp türleri ve süre açısından örnekler karşılaştırıldığında anlamlı korelasyonlar mevcuttu (p <0.001).

Sonuç: Bu çalışmanın sonucunda, kötüye kullanılan ilaçların analizinde cam tüpler yerine plastik tüplerin kullanılabileceği, oda sıcaklığında dört saat saklandıktan sonra madde konsantrasyonlarında anlamlı bir değişimin olmadığı sonucu çıkarılabilir.

Anahtar kelimeler: İdrar, Madde analizleri, Yasadışı madde analizleri

INTRODUCTION

Drug abuse is considered one of the major preventable public health and safety problems in the world. The fight against drugs is among the priority goals of countries, and determining substance use through drug screening is one of the most important elements in this fight (1). Drug screening through urinalysis is the most suitable and widely accepted tool for rapidly detecting potential drug use (2). Immunochemical methods are frequently used in medical laboratories for urine drug screening. Urine drug screening by immunoassay in routine laboratories is an automated, simple, rapid, semi-quantitative, and cost-effective analysis. A single urine sample can be used to analyze all desired stimulants and drugs by immunoassays.

The substances that test positive in drug screening analysis are then subjected to confirmatory analysis upon administrative or judicial request. The confirmatory analysis is performed using gas chromatography-mass spectrometry (GC-MS) or liquid chromatography-mass spectrometry (LC-MS/MS), providing quantitative detection with the same urine specimen (3,4). The urine specimen collection is simple, non-invasive, and allows for a wide detection window for most drugs and/or drug metabolites (2). The sample collection container must be suitable for the specimen to ensure it does not affect analytical test results.

Recently, plastic collection tubes have started to replace glass tubes in many laboratories. These disposable plastic tubes are cheaper than glass tubes and are also suitable for storage at low temperatures and transportation. However, depending on the physicochemical properties, some drug analytes tend to adhere to plastic surfaces (5). Plastic tubes have been shown to

influence the measured concentrations or stabilities of various therapeutic drugs and peptide hormones in the blood (6,7,8).

This study aims to investigate the impact of urine collection tubes on drug abuse tests and evaluate whether plastic tubes can serve as an alternative to glass tubes.

METHODS

The urine drug analysis of 80 drug abusers, aged between 18-65 years, who applied to the Alcohol and Substance Abuse Treatment and Research Centre (AMATEM), affiliated with the Psychiatry Clinic of the University Hospital, was included in this study. Urine samples were collected according to the quality and safety requirements outlined in the latest regulation of the Department of Medical Laboratory Services, Ministry of Health in Turkey (9). To address the problem of tampering with the urine specimens, the fresh urine samples were collected under observation, separated into glass and plastic tubes (Vacusera Urine Tube No Additive) simultaneously, and transferred to the laboratory for analysis.

We used polypropylene tubes, which are a type of plastic commonly used in laboratories due to their low adsorption properties, chemical resistance, and durability. Drug abuse analysis began after the specimen integrity tests, such as creatinine levels, specific gravity, and pH values, were used to detect substitution, adulteration, or dilution (10). To investigate the effect of time, 32 samples were stored at room temperature for 4 hours without any preservative and reanalyzed.

The levels of amphetamines (AMP), benzodiazepines (BNZ), cocaine or metabolites (COC), opiates (OPI), and cannabinoids (THC) in urine were measured using Roche Cobas c 501 auto analyzers (Roche Diagnostics GmbH, Mannheim, Germany) with the kinetic interaction of microparticles in solution (KIMS) method. This is an in vitro diagnostic test for semi-quantitative and qualitative measurements. The cut-off values for positivity were defined as AMP > 500 µg/L, BNZ > 300 µg/L, COC > 150 µg/L, OPI > 2000 µg/L, and THC > 50 µg/L (9).

The daily internal quality control results (negative and positive levels according to the cut-off values) were acceptable for any drug test (11). The external quality assessment (EQA) was also acceptable, with positive or negative results matching the target positive or negative results of each drug test in external quality control material (Oneworld Accuracy EQA Program, Vancouver, Canada). Written consent was routinely obtained from all participants. The study was approved by the Research Ethics Commission of the University (Approval No. 60116787-020/2372, Date: 25.12.2018), with respect to the ethical standards of the Declaration of Helsinki.

Statistical Analysis

IBM SPSS Statistics 22 (SPSS Inc., Chicago, Ill, USA) program was used for statistical analysis. The suitability of the parameters to the normal distribution was evaluated by Kolmogorov-Smirnov and Shapiro-Wilks tests. In addition to descriptive statistical methods (minimum, maximum, mean, standard deviation, median frequency), Student's t-test was used for comparisons of normally distributed parameters between two groups, and Mann Whitney U test was used for comparisons of non-normally distributed parameters between two groups. The Chi-Square test and Fisher's Exact Chi-Square test were used to compare qualitative data. A p-value less than 0.05 was set as the statistical significance level. Bland–Altman analysis and correlation coefficients were used to evaluate the interchangeability between plastic and glass tube (as a reference) results.

RESULTS

The temperatures of the samples were between 33–37 °C. The urine integrity tests were performed, and all results were within acceptable ranges [(The acceptable ranges:

pH: 3–11, specific gravity: 1003–1035, creatinine: 20–200 mg/dL, nitrite: negative (Cut-off: 500 mg/L)] (9,12,13). Next, five different drug abuse tests were performed on 80 paired samples drawn into glass and plastic tubes.

Among the 80 patients' urine samples, positive results were as follows: BNZ (n=6), COC (n=1), AMP (n=5), OPI (n=9), and THC (n=5) in both glass and plastic tubes. In the 32 samples stored at room temperature for 4 hours, the positivity or negativity of results was not affected.

Paired t-tests of 80 samples showed no statistically significant differences between glass and plastic tubes for AMP, COC, BNZ, and THC. However, a statistically significant difference (p= 0.005) was found for OPI (Table 1). For stored samples (n= 32), only BNZ showed a statistically significant difference (p= 0.037), with BNZ concentrations being higher in plastic tubes (Table 2). Nonetheless, these differences did not change the positivity or negativity of the samples and were determined to be clinically insignificant.

There was no statistically significant difference between the 0-minute and 4-hour measurements in either plastic or glass tubes at room temperature ($p > 0.05$). Both types of tubes showed similar results for all analytes, and the 4-hour storage duration at room temperature did not significantly affect the test outcomes (Table 3).

Spearman correlation coefficients (r) comparing tube types and storage time for all tests showed statistically significant agreement ($p< 0.001$) with strong correlations. Intraclass correlation coefficients (ICC) for tube comparisons are presented in Table 4. Bland-Altman plots for all tests are provided in Figure 1.

Table 1. Comparison data of glass vs. plastic collection tubes on test results

Table 1. Cam ve plastik toplama tüplerinin test sonuçları üzerindeki etkilerinin karşılaştırılması.

	Glass (n= 80)		Plastic (n= 80)		
Tests (µg/L)	**Median**	**Mean**	**Median**	**Mean**	**p-value**
Benzodiazepines (BNZ)	38 (0 - 3472)	165.08 ± 503.58	43.5 (0 - 3460)	166.99 ± 501.14	0.304
Cocaine (COC)	13 (0 - 1301)	28.98 ± 144.47	14 (0 - 1332)	29.41 ± 147.87	0.961
Amphetamines (AMP)	66.5 (0 - 552)	98.03 ± 118.1	69 (0 - 561)	100.54 ± 117.11	0.247
Opiates (OPI)	29.5 (0 - 239184)	3306 ± 26721.71	21.5 (0 - 211463)	2955.65 ± 23625.39	0.005*
Cannabinoids (THC)	4.5 (0 - 366)	15.51 ± 47.92	4 (0 - 350)	15.29 ± 46.83	0.625

Statistically significant results (<0.05) are indicated with an asterisk (*).

Table 2. Comparison data of glass vs. plastic collection tubes after stored for 4 hours at room temperature on test results

Table 2. Cam ve plastik toplama tüplerinin, oda sıcaklığında 4 saat saklandıktan sonraki test sonuçları üzerindeki etkilerinin karşılaştırılması

	Glass (n= 32)		Plastic (n= 32)		
Tests (µg/L)	**Median**	**Mean**	**Median**	**Mean**	**p-value**
Benzodiazepines (BNZ)	8 (0 - 1980)	165.75 ± 464.09	15.5 (0 - 1994)	174.5 ± 463.68	0.037*
Cocaine (COC)	12.5 (0 - 34)	11.63 ± 9.64	7 (0 - 32)	10.81 ± 10.86	0.513
Amphetamines (AMP)	67 (5 - 527)	99.44 ± 122.33	67 (0 - 520)	102.66 ± 118.69	0.61
Opiates (OPI)	9.5 (0 - 5317)	500.94 ± 1328.61	20 (0 - 5289)	502.47 ± 1320.97	0.603
Cannabinoids (THC)	5.5 (0 - 179)	12.91 ± 31.99	6 (0 - 155)	12.63 ± 27.88	0.359

Statistically significant results (<0.05) are indicated with an asterisk (*)

Table 3. Comparison of 0-minute and 4-hour Measurements in Plastic and Glass Tubes at Room Temperature
Table 3. Plastik ve cam tüplerde oda sıcaklığında 0. dakika ve 4. saat ölçümlerinin karşılaştırılması

	Glass (n:32)				
	0-minute		4 hours at RT		
Tests (µg/L)	**Median**	**Mean**	**Median**	**Mean**	**p**
Benzodiazepines (BNZ)	10 (0 - 1974)	165.08 ± 490.48	8 (0 - 1980)	165.75 ± 464.09	0.966
Cocaine (COC)	12.4 (0 - 39)	12 ± 10.26	12.5 (0 - 34)	11.63 ± 9.64	0.871
Amphetamines (AMP)	66.5 (0 - 552)	100.03 ± 127.64	67 (5 - 527)	99.44 ± 122.33	0.794
Opiates (OPI)	10.5 (0 - 5327)	489.42 ± 955.43	9.5 (0 - 5317)	500.94 ± 1328.61	0.853
Cannabinoids (THC)	4.5 (0 - 163)	12.51 ± 42.92	5.5 (0 - 179)	12.91 ± 31.99	0.787
	Plastic (n:32)				
Benzodiazepines (BNZ)	24 (0 - 1951)	175 ± 476.94	15.5 (0 - 1994)	174.5 ± 463.68	0.995
Cocaine (COC)	10 (0 - 39)	11.56 ± 8.54	7 (0 - 32)	10.81 ± 10.86	0.85
Amphetamines (AMP)	69 (0 - 561)	108.54 ± 117.11	67 (0 - 520)	102.66 ± 118.69	0.868
Opiates (OPI)	21.5 (0 - 5475)	515 ± 1419.09	20 (0 - 5289)	502.47 ± 1320.97	0.992
Cannabinoids (THC)	4 (0 - 167)	11.80 ± 19.63	6 (0 - 155)	12.63 ± 27.88	0.897

Table 4. Intraclass correlation coefficients of drug abuse tests
Table 4. Uyuşturucu madde testlerinin sınıf içi korelasyon katsayıları

	The intraclass correlation for the 5 tests in 2 tubes at each time interval		
	Intraclass correlation coefficients (ICC)	**95% confidence interval (95% CI)**	**p-value**
BZN plastic-glass	1	1.000 - 1.000	<0.001*
4 hours at RT	0.999	0.998 - 0.999	<0.001*
AMP plastic-glass	0.994	0.991 - 0.996	<0.001*
4 hours at RT	0.993	0.986 - 0.997	<0.001*
COC plastic-glass	0.999	0.999 - 1.000	<0.001*
4 hours at RT	0.873	0.742 - 0.937	<0.001*
OPI plastic-glass	0.996	0.994 - 0.981	<0.001*
4 hours at RT	1	1.000 - 1.000	<0.001*
THC plastic-glass	0.999	0.998 - 0.999	<0.001*
4 hours at RT	0.994	0.987 - 0.997	<0.001*

Statistically significant results (<0.05) are indicated with an asterisk (*). AMP: Amphetamines. BNZ: Benzodiazepines. COC: Cocaine or metabolites. OPI: Opiates. THC: Cannabinoids. RT: Room temperature

DISCUSSION

In recent years, plastic sampling tubes have increasingly replaced glass tubes. Plastic tubes are inexpensive, break-resistant, safe for laboratory employees, and suitable for freezing samples (6). However, plastic tubes have been reported to cause adsorption or degradation of some analytes (14). Numerous studies have investigated the stability of therapeutic drugs in plastic or glass tubes (9,15); however, for drug abuse tests, there are only a few published reports (16,17,18). We compared the effects of glass versus plastic urine collection tubes on the results of five drug abuse tests and found no clinically significant differences and strong correlations between results in plastic and glass.

Previous studies reported that cannabinoids are hydrophobic molecules subject to adsorption to solid surfaces from aqueous solutions such as urine. Glass is reportedly an optimal material for the handling of cannabinoids (16,17). Bruno et al. evaluated the storage conditions of cannabinoids in plastic tubes versus glass tubes and found no significant difference in concentration between glass and plastic tubes at −20°C and −80°C. Furthermore, the study reported no degradation/adsorption in the first week of storage at +4°C (18). Our findings also

showed that glass or plastic tubes do not affect ~~the~~ cannabinoid levels, and we found no difference in the THC concentrations even after storage at room temperature for 4 hours in both plastic and glass tubes. The possible reason for findings differing from previous studies may be advances in the manufacturing of plastic tubes that reduce analyte adsorption or degradation of THC.

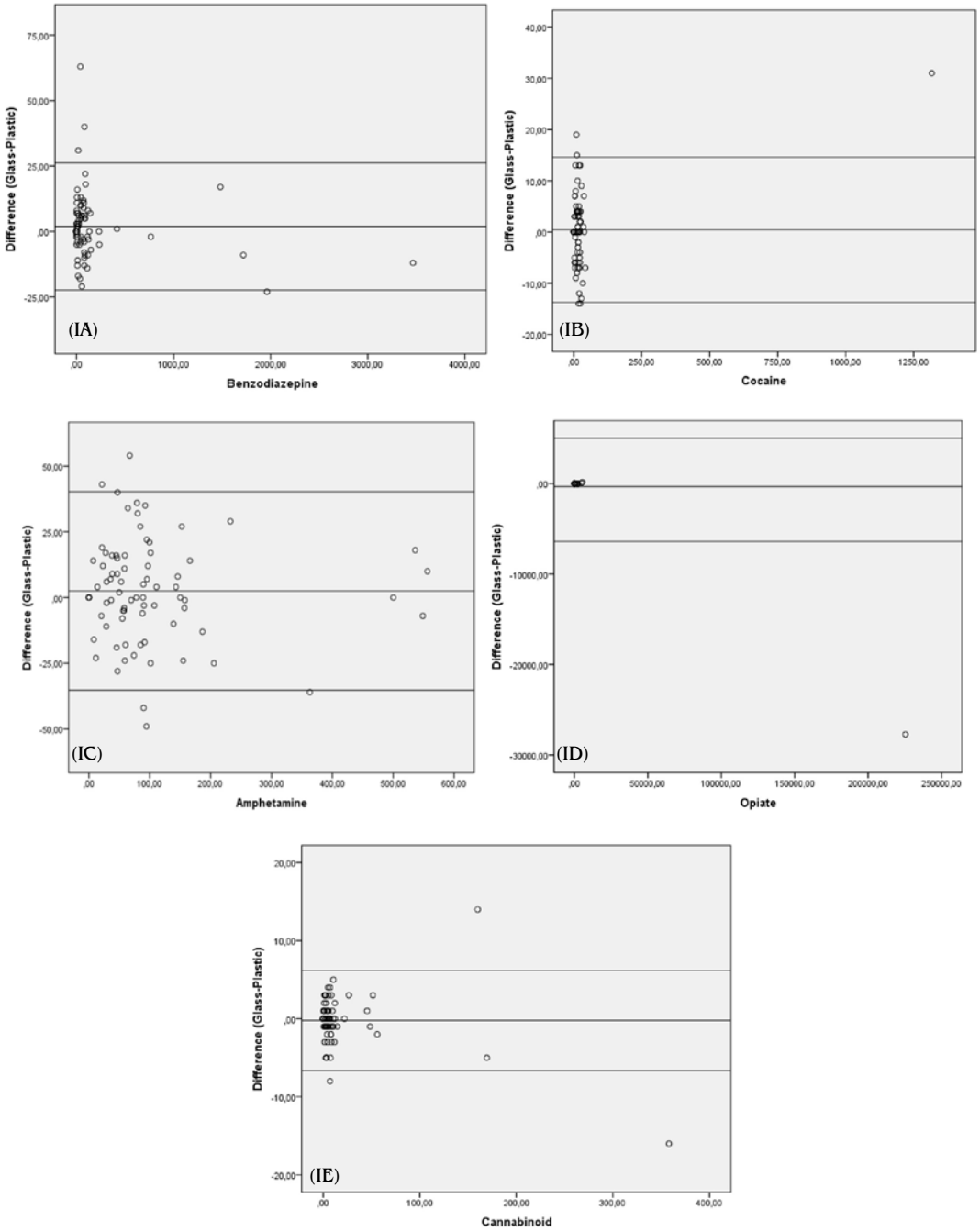

Figure 1. The Bland-Altman plots show the differences (y-axis, µg/L) and average values (x-axis, µg/L) for amphetamines (IA), benzodiazepines (IB), cocaine or metabolites (IC), opiates (ID), and cannabinoids (IE) between paired samples drawn into glass and plastic tubes

To our knowledge, this study is the first to compare the effects of glass versus plastic urine collection tubes on drug abuse tests of AMP, COC, BNZ, and OPI. Our findings showed no differences between glass and plastic tubes for AMP, BNZ, and COC levels. A statistically significant difference was found for OPI (Table 1), but this difference did not change the sample's positivity or negativity results based on the cut-off value. There were isolated statistically significant differences between glass and plastic tubes after 4 hours of storage for BNZ (Table 2), were an increase in BNZ concentration was observed. However, this difference was clinically insignificant. Although there were numerical differences in some drug results, it is important to emphasize that all measurements were compatible with each other based on the cut-off values. Literature indicates that all immunoassays do not perform equally well for some drugs (19). While quantitative methods may provide more accurate results, these methods require greater expertise, have longer processing times, and are costly (20). Regarding the statistically significant difference observed for opiates, we recommend that laboratories consider further validation studies, particularly using more precise analytical techniques such as GC-MS (Gas Chromatography-Mass Spectrometry), to ensure that differences between collection tube materials do not influence clinical decisions.

A possible limitation of our study is that most of the test results were below the cut-off value. Further studies with more abnormal samples should be conducted. Among the abnormal results in this study, there were no significant differences between glass and plastic collection tubes that changed the clinical decision. Additionally, as we conducted the study in affiliation with AMATEM, we faced difficulties in obtaining positive samples, which limited the robustness of our analysis.

CONCLUSION

Our study demonstrated no clinically significant differences between plastic and glass urine collection tubes for drug abuse tests, including AMP, COC, BNZ, OPI, and THC, even after short-term storage. These findings suggest that plastic tubes are a suitable alternative to glass tubes for drug abuse testing, though further studies with larger sample sizes and more precise testing methods, such as GC-MS are recommended to confirm these results.

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