

THE EFFECT OF SOME ADULTERANTS ON DRUG ABUSE DETECTION BY IMMUNOASSAY TEST STRIPS

Reham Hassan El- Farouny ^a, Heba Allah Ali Mabrouk ^b
and Nagwa Mahmoud Habib ^a

^a Forensic Medicine and Clinical Toxicology Department, Faculty of Medicine- Menoufia University, Egypt

^b Forensic Medicine & Clinical Toxicology - Faculty of Medicine - Kafrelsheikh University, Egypt

Corresponding author: Reham Hassan El-Farouny.

E-mail address: dr_reham2004@yahoo.com

ABSTRACT

Background: The use of immunoassays for drug screening has increased due to their sensitivity towards target analytes. Due to their potential to interfere with drug screening tests and provide false findings, adulterants pose a new challenge in the detection of drug abuse. **Objectives:** The current study aims to evaluate the effect of some adulterants on the detection of drug abuse in urine tested by immunoassay test strips and the effect of the adulterants on the validity of the results of these tests. **Methodology:** Urine samples tested positive by immunoassay test strips for one of the five abused drugs (tramadol, cannabis, morphine, benzodiazepines (BDZ), and amphetamines) were used. With the help of Indiko (Thermoscientific fully automated urine enzyme immunoassay), we chose two different concentrations of each drug, the first is just above the cutoff level of test strips, and the other is higher than the double cutoff. Four adulterants (vinegar, bleach, Visine eye drops, and water) were tested for their ability to generate false negative results for the chromatographic immunoassay test strips. Each adulterant was added to a urine sample containing 1 of 5 different drugs at fixed concentrations. Adulterants were also added to negative control samples to reveal how integrity criteria (Ph) and specific gravity (SG) were affected. **Results:** Out of the 4 adulterants, vinegar generated the most false negatives. Bleach turned both low and high concentrations of positive cannabis samples into negative ones. Visine eye drops masked the positivity of the low-concentration cannabis sample. Water was effective in concealing the positivity of the low-concentration BDZ sample. **Conclusion:** A positive urine sample that includes drugs may nevertheless be examined and declared "clean and free of drugs" after passing a routine screening procedure. We recommend that drug screening tests be frequently combined with adulterant test strips to guarantee that the integrity of the specimen has not been compromised before drug testing.

Keywords: Adulteration, drug abuse, urine immunoassay test strips

INTRODUCTION

Substance misuse poses a severe risk to the public's health all over the world, and also in Egypt. It impacts young people throughout their productive years, causing a variety of issues such as poor social adjustment, reduced productivity, and unemployment. Recent studies have shown a rise in the frequency of cannabis and tramadol usage (Abdel Ati et al., 2020).

A crucial tool in the country's war on substance abuse is urine testing. These drug tests are used for inmate deterrence,

probation control, post-accident evaluation, and pre-employment screening (Jaffee et al., 2007).

The most popular method of drug testing is urine analysis because, contrary to other biological matrices like blood or hair, it provides a wider detection window for illegal substances, costs less to conduct, especially for bigger organizations, and requires less sample preparation. However, the potential for sample adulteration, or replacement, is a drawback of all urine drug tests. (Olivieri et al., 2018).

Drug testing is divided into two stages; screening and confirmatory. Immunoassay tests have traditionally been used as the initial screening, these tests can give an idea about the drugs or their metabolite's presence in the sample. Besides that, it can provide a reasonable turnaround time with little resources and labor (Al-khayal et al., 2017).

Adulterants present a new obstacle in the detection of abused drugs as they could disrupt the drug screening test, resulting in incorrect results (Abdelati et al., 2020). Adulteration is defined as any action taken by a person to intentionally interfere with the collection, transportation, or analysis of a specimen to represent a false negative test result (Olivieri et al., 2018). On the other hand, it may be successfully detected via a specimen validity test (SVT), which often comprises a visual inspection of the physical properties of urine, pH, creatinine level, and specific gravity (Vikingsson et al., 2022).

The literature claims that drug users alter samples in three different ways to produce false-negative results. The first is to drink a lot of fluids and/or take diuretics to significantly dilute urine. The second is substituting a sample of urine devoid of drugs for their own (synthetic or obtained from another person) (Mizrak, 2019). However, drug users turn to the third method, which is easy, by adding urine adulterating substances to achieve a falsely negative result (adulteration in situ and in vitro). Acids, alkalis, surfactants, and oxidizing agents are just a few of the chemical compounds that can have this influence. Many other new chemicals have even been developed to evade drug tests that are now on the market (Rajšić et al., 2020).

Therefore, the current study's objective is to assess how various adulterants affect the ability to identify drug abuse in urine tested by immunoassay test strips and the effect of the adulterants on the validity of the results.

MATERIALS AND METHODS:

After receiving approval from the Faculty of Medicine's Ethics Committee

(Menoufia University number: 6/2022FORE14), this case-control research was carried out at the Forensic and Analytical Chemistry Lab of the Forensic and Clinical Toxicology Department.

1) Urine samples: Urine samples used in this study were part of cases submitted to our toxicological laboratory for drug screening analysis. Accepted samples (inclusion criteria):

Samples with a volume of more than 20 ml of urine, were voided in a clean, dry, labeled plastic container without any preservative. The samples that tested positive by immunoassay test strip for one of the following drugs; tramadol, cannabis, morphine, benzodiazepines (BDZ), or amphetamines. These five drugs were chosen as we found them the most common among the represented samples. Drug-free urine samples were collected from healthy donors to be used as a control sample in urine sample integrity tests. Urine specimens were transported to the Forensic and Analytical Chemistry Lab. Every aspect of specimen handling, analysis, security verification, and documentation of the chain of custody was followed in the present study.

At first, all urine samples were examined for the physical properties of normal urine as color, odor, and visible precipitate or turbidity. Samples with abnormal physical properties were excluded.

A screening test for common substance abuse was done using the "ACCURATE" Multi-Drug Rapid Test Panel (Fig 1). If the immunoassay test result was negative; it means that the drug level is below the cutoff and the specimen was excluded. If the immunoassay result was positive, we further established the level of the drug or drug metabolite by using Indiko Thermo scientific fully automated urine enzyme immunoassay; which is an immunoassay for qualitative and semi-quantitative drug analysis in human urine with automated clinical chemistry analyzer (Thermo Scientific Indiko Chemistry Analyzer manual, 2013)(Fig 2).

Two samples with different concentrations for each drug were chosen

with the help of Indiko the first was a low concentration that was just above the cutoff level of test strips, and the second was higher than double the cutoff level of test strips, as shown in **table (1)**. Negative control samples and positive samples of the different drugs were divided into 4 subgroups; every subgroup was subjected to one adulterant as follows:

1- Group I (control): Negative samples were divided into 4 subgroups; each one was 5 ml urine. Each adulterant is added to one subgroup (to check the effect of adulterants on urine integrity test by test strips for urine sample integrity) as follows:

I-1: adulterated with vinegar

I-2: adulterated with a bleaching agent

I-3: adulterated with Visine eye drops

I-4: adulterated with water.

2- Group II: Tramadol-positive samples. Group II includes 2 subgroups according to concentration: IIa and IIb, which were further divided into 4 subgroups to check the effect of adulterants on the results of urine drug screening test (UDST); each sample is 5 ml as follows:

IIa: Samples with tramadol concentration just above cut off level of test strips (134 ng/ml):

IIa-1: adulterated with vinegar

IIa-2: adulterated with a bleaching agent

IIa-3: adulterated with Visine eye drops

IIa-4: adulterated with water

IIb: samples with tramadol concentrations higher than the double cut-off level of test strips (450 ng/ml):

IIb-1: adulterated with vinegar

IIb-2: adulterated with a bleaching agent

IIb-3: adulterated with Visine eye drops

IIb-4: adulterated with water

3- Group III: Cannabis-positive samples. Group III includes 2 subgroups according to concentration: IIIa and IIIb, which were further divided into 4 subgroups to check the effect of adulterants on the results of UDST (each sample is 5 ml) as follows:

IIIa: samples with cannabis concentration just above cut off level of test strips (27 ng/ml):

IIIa-1: adulterated with vinegar

IIIa-2: adulterated with a bleaching agent

IIIa-3: adulterated with Visine eye drops

IIIa-4: adulterated with water

IIIb: samples with cannabis concentrations higher than the double cut-off level of test strips (99 ng/ml):

IIIb-1: adulterated with vinegar

IIIb-2: adulterated with a bleaching agent

IIIb-3: adulterated with Visine eye drops

IIIb-4: adulterated with water

4- Group IV: BDZ positive samples. Group IV includes 2 subgroups according to concentration: IVa and IVb, which were further divided into 4 subgroups to check the effect of adulterants on the results of UDST (each sample is 5 ml) as follows:

IVa: samples with BDZ concentration just above cut off level of test strips (218 ng/ml):

IVa-1: adulterated with vinegar

IVa-2: adulterated with a bleaching agent

IVa-3: adulterated with Visine eye drops

IVa-4: adulterated with water

IVb: samples with BDZ concentrations higher than the double cut-off level of test strips (500 ng/ml):

IVb-1: adulterated with vinegar

IVb-2: adulterated with a bleaching agent

IVb-3: adulterated with Visine eye drops

IVb-4: adulterated with water

5- Group V: Amphetamine-positive samples. Group V includes 2 subgroups according to concentration: Va and Vb, which were further divided into 4 subgroups to check the effect of adulterants on the results of UDST (each sample is 5 ml) as follows:

Va: samples with amphetamine concentration just above cut off level of test strips (400 ng/ml):

Va-1: adulterated with vinegar

Va-2: adulterated with a bleaching agent

Va-3: adulterated with Visine eye drops

Va-4: adulterated with water

Vb: samples with amphetamine concentrations higher than the double cut-off level of test strips (900 ng/ml):

Vb-1: adulterated with vinegar

Vb-2: adulterated with a bleaching agent

Vb-3: adulterated with Visine eye drops

Vb-4: adulterated with water

6- Group VI: Morphine-positive samples. Group VI includes 2 subgroups according to concentration: VIa and VIb, which were further divided into 4 subgroups to check the effect of adulterants on the results of UDST (each sample is 5 ml) as follows:

VIa: samples with morphine concentration just above cut off level of test strips (150 ng/ml):

VIa-1: adulterated with vinegar

VIa-2: adulterated with a bleaching agent

VIa-3: adulterated with Visine eye drops

VIa-4: adulterated with water

VIb: samples with morphine concentrations higher than the double cut-off level of test strips (300 ng/ml):

VIb-1: adulterated with vinegar

VIb-2: adulterated with a bleaching agent

VIb-3: adulterated with Visine eye drops

VIb-4: adulterated with water

2) Urine Drug Screening Tests (UDST):

"ACCURATE" Multi-Drug Rapid Test Panel is a rapid chromatographic immunoassay for the qualitative detection of common substances of abuse: Tramadol, cannabis, morphine, BDZ, barbiturates, amphetamines, and cocaine. We purchased the test strips from Hangzhou All Test Biotech Co., Ltd., which supplies laboratories and pharmacies.

These tests are qualitative, so a positive result means that the drug that is being screened for is most likely present in urine at a concentration above the cut-off level (**table 1**).

Test interpretation is as follows in Fig (1):

Negative: Two lines appear. One red line should be in the control region (C), and another apparent red or pink line in the test region (T).

Positive: One red line appears in the control region (C). No line appears in the test region (T).

Invalid: Control line fails to appear; insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure.

3) Test strips for urine sample integrity check:

Negative urine samples with adulterants were tested for integrity by Medi-Test Combi 10 SGL (MACHEREY-NAGEL GmbH & Co. Germany), to measure how pH and specific gravity (SG) values were affected. pH normal range is between 5 - 9, SG normal range: 1.000-1.030. Dipsticks were photographed together with the manufacturer-provided color guide, and the results were determined visually.



Figure (1): Immunoassay test strip interpretation the test is positive for cannabis (one line at the C region), as shown by the black arrow, while it is negative for the rest of the drugs (two lines, one at the C and the other at the T regions).



Figure (2): Indiko thermoscientific fully automated urine enzyme immunoassay. (Thermo Scientific Indiko Chemistry Analyzer manual, 2013).

4) Adulteration and testing:

Adulterants used were acids (vinegar 5%), alkalis (bleaching liquid sodium hypochlorite (NaClO) less than 5%), surfactants (Visine eye drops), and dilution by tap water.

The amount of each adulterant added to urine samples for all groups was as follows: 0.3 ml of vinegar/ 20 ml urine, 0.2 ml of bleach, 0.4 ml of Visine eye drops, and 2 ml of water, according to **Rajšić et al., 2020**, where excessive amounts of these substances alter the physical look of urine; As color (it has turned extremely light), odor (has the scent of a chemical component), or consistency (it has turned foamy or turbid).

RESULTS

As shown in **Figure 3**, the results of urine tests using Integrity urine strips

testing the pH and specific gravity on negative control samples, and samples after the addition of adulterants in group I revealed that there was some sort of manipulation in the urine samples, where vinegar changed the pH into a yellow color (acidic), bleach turned it into a green color (alkaline), and water changed specific gravity while Visine had no effect.

As described in **Table 1**, the cutoff level of each drug of the five chosen drugs (tramadol, cannabis, BDZ, amphetamines, and morphine) according to the manufacture of test strip immunoassay was mentioned, as well as the two concentrations of samples that were selected with the help of Indiko (the low concentration is just above the cutoff level of test strips for each drug, and the second is higher than the double its cutoff level) that were tested.

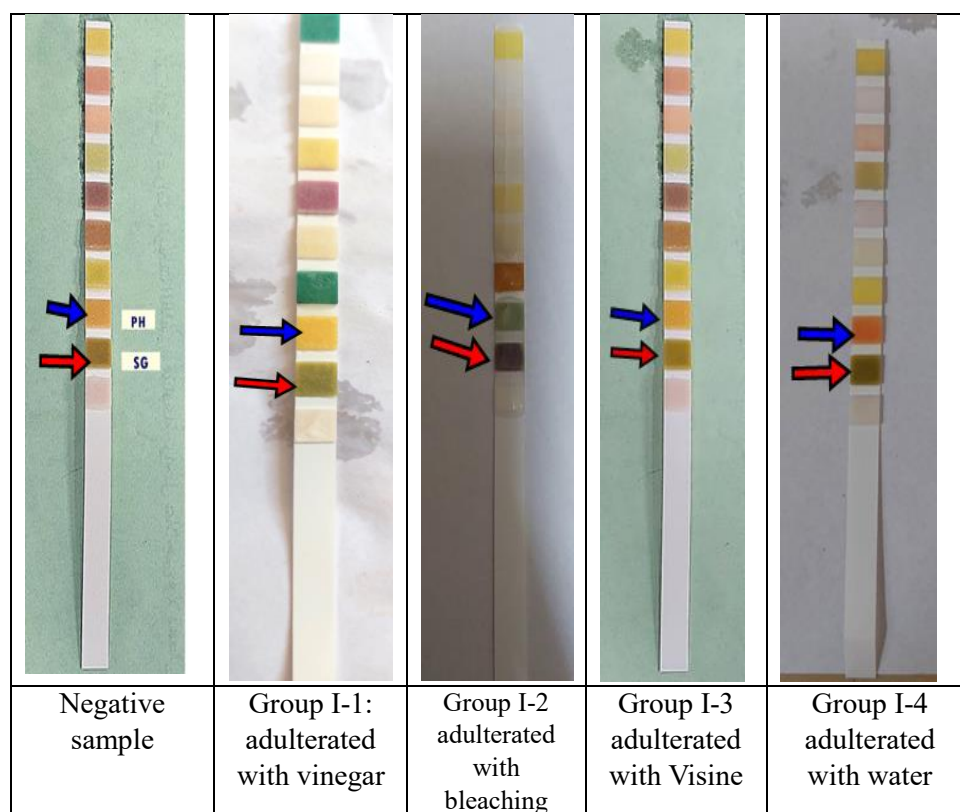


Figure (3): Integrity urine strips tested of PH (the blue arrow) and specific gravity (the red arrow) on negative control samples and control samples after the addition of adulterants in group I (1, 2, 3, and 4)

Table (1): Drug concentrations of the selected samples as measured by Indiko:

Drug	The cut-off level of the test strip (ng/ml)	Sample a (used concentration just above cut-off level) (ng/ml)	Sample b (used concentration higher than double cut-off level) (ng/ml)
Tramadol	100	134	450
Cannabis	20	27	99
BDZ	200	218	500
Amphetamines	300	400	900
Morphine	100	150	300

The effects of several adulterants on urine samples containing tramadol are shown in **Table 2 and Figures 4 and 5**; where vinegar was able to mask the positive findings and produce false

negative results in both groups (II-a 1 and II-b 1). However, adding bleaching liquid, Visine, or water did not succeed in preventing an immunoassay test strip from detecting tramadol.

Table (2): Urine drug immunoassay test results obtained after adulteration of group II urine samples (positive for tramadol):

Group II a (134 ng/ml)	Result	Group II b (350 ng/ml)	Result
II-a 1	-	II-b 1	-
II-a 2	+	II-b 2	+
II-a 3	+	II-b 3	+
II-a 4	+	II-b 4	+



Figure (4): Urine drug immunoassay test results of group IIa urine samples (positive for tramadol)



Figure (5): Urine drug immunoassay test results of group IIb urine samples (positive for tramadol)

Table 3, figures 6 and 7; illustrate the impact of some adulterants on urine samples containing cannabis. We found that in both groups (III-a1, 2, and III-b1, 2), vinegar and bleaching solutions were able to obscure the positive results and produce false negative results. While

Visine obscured the positive results in the low concentration only in group (IIIa3) and didn't affect the results in the high concentration in group (IIIb3), in contrast, water was not able to change the immunoassay test strip to identify the active ingredient in cannabis (THC).

Table (3): Urine drug immunoassay test results obtained after adulteration of group III urine samples (positive for cannabis):

Group III a (35 ng/ml)	Result	Group III b (100 ng/ml)	Result
III-a 1	-	III-b 1	-
III-a 2	-	III-b 2	-
III-a 3	-	III-b 3	+
III-a 4	+	III-b 4	+



Figure (6): Urine drug immunoassay test results of group IIIa urine samples (positive for cannabis)



Figure (7): Urine drug immunoassay test results of group IIIb urine samples (positive for cannabis)

Tables 4 and Figures 8 and 9 show how different adulterants affect urine samples containing BDZ. Vinegar was able to mask the positive results in both groups (IVa and IVb). Bleaching solution and Visine could not conceal the immunoassay test strip's ability to detect BDZ in samples

of urine either in low or high concentrations, while dilution with water was capable of masking the positive results in the low concentration group (IVa 4), but didn't change the positivity of the test strips to BDZ in the high concentration group (IVb 4).

Table (4): Urine drug immunoassay test results obtained after adulteration of group IV urine samples (positive for BDZ):

Group IV a (250 ng/ml)	Result	Group IV b (450 ng/ml)	Result
IV-a 1	-	IV-b 1	-
IV-a 2	+	IV-b 2	+
IV-a 3	+	IV-b 3	+
IV-a 4	-	IV-b 4	+

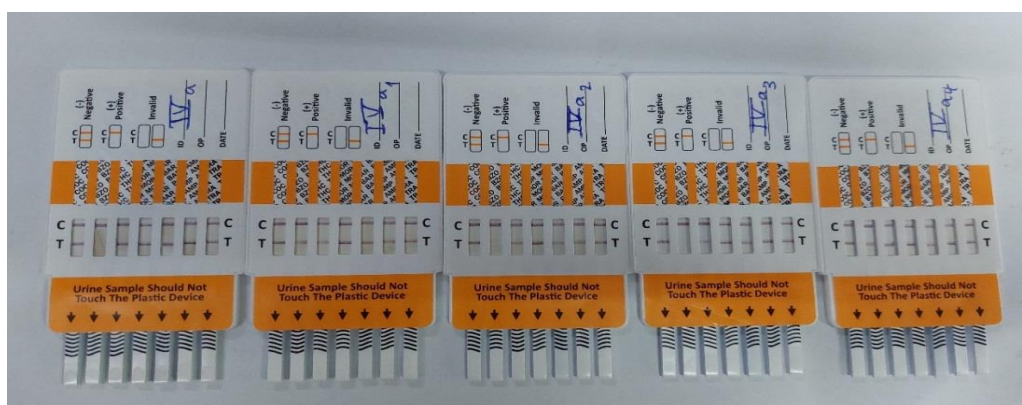


Figure (8): Urine drug immunoassay test results of group IVa urine samples (positive for BDZ)



Figure (9): Urine drug immunoassay test results of group IVb urine samples (positive for BDZ)

The effects of several adulterants on urine samples containing amphetamine are shown in **Table 5** and **Figures 10** and **11**, where vinegar was able to mask positive findings and produce false

negative results in both groups (**V-a 1** and **V-b 1**). However, bleaching liquid, Visine, or water did not succeed in masking the positive results of the immunoassay test strip from detecting amphetamine.

Table (5): Urine drug immunoassay test results obtained after adulteration of group V urine samples (positive for amphetamine):

Group V a (330 ng/ml)	Result	Group V b (686 ng/ml)	Result
V-a 1	-	V-b 1	-
V-a 2	+	V-b 2	+
V-a 3	+	V-b 3	+
V-a 4	a+	V-b 4	+



Figure (10): Urine drug immunoassay test results of group Va urine samples (positive for amphetamine)

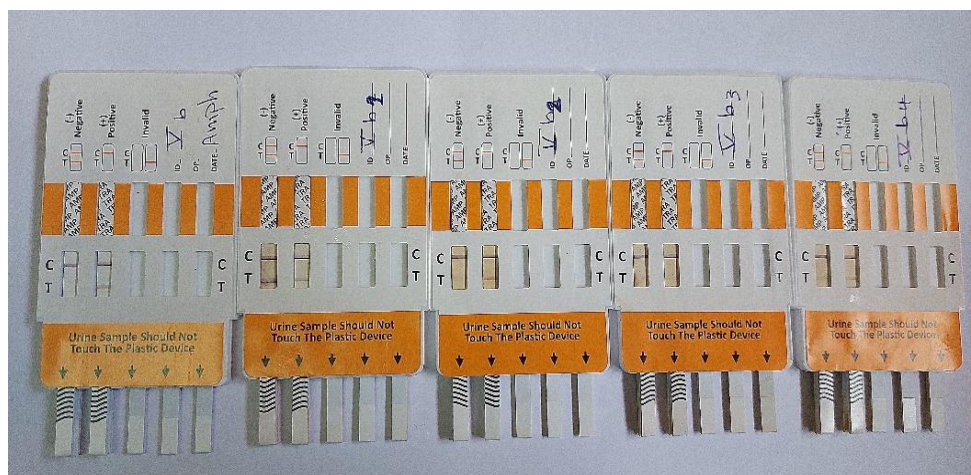


Figure (11): Urine drug immunoassay test results of group Vb urine samples (positive for amphetamine)

The effects of several adulterants on urine samples containing morphine are shown in **Table 6** and **Figures 12** and **13**, where vinegar was able to produce false negative results in both groups (**VIa 1** and

VIb 1). However, bleaching liquid, Visine, or water did not succeed in changing the positive results of morphine urine samples tested by immunoassay test strips in both concentrations.

Table (6): Urine drug immunoassay test results obtained after adulteration of group VI urine samples (positive for morphine):

Group VI a (150 ng/ml)	Result	Group VI b (282 ng/ml)	Result
VI-a 1	-	VI-b 1	-
VI-a 2	+	VI-b 2	+
VI-a 3	+	VI-b 3	+
VI-a 4	+	VI-b 4	+



Figure (12): Urine drug immunoassay test results of group VIa urine samples (positive for morphine)

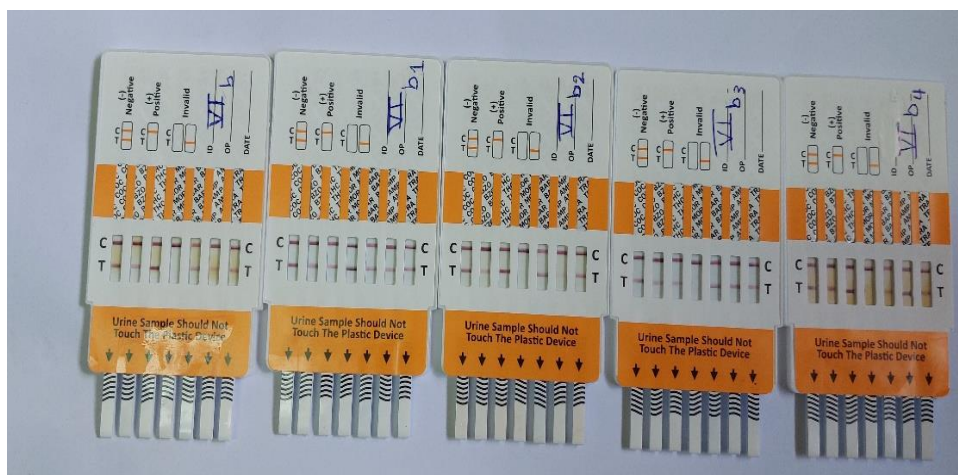


Figure (13): Urine drug immunoassay test results of group VIb urine samples (positive for morphine)

DISCUSSION

Due to their widespread availability in houses, bathroom cabinets, and markets, as well as their popularity as *in vitro* adulterants, we chose common household products (vinegar and bleach) for the current investigation. Vinegar is an example of an acidic compound, and sodium hypochlorite bleach is an example of an alkaline compound. We also chose Visine eye drops as an example of a popular over-the-counter pharmaceutical medicine that is widely known among addicts as a good adulterant. Visine is an example of a surfactant substance.

Despite the high sensitivity of immunoassay, a negative drug result for urine samples does not necessarily indicate that no drug was present. It's possible that the drug concentration fell below the threshold for detection in the lab assay (Hadland and Levy, 2016). Detection of drugs in a urine sample depends on many factors, mainly its concentration in urine, which is affected by the time of sample collection. For instance, early morning specimen collection has the maximum concentration; therefore, the samples will have higher drug concentrations (Karen et al., 2017). Additionally, the drug's or its metabolites' concentration in urine depends on the time since the last dose. Compared to chronic cannabis and BDZ use, which

may be detectable for up to 30 days, amphetamines and cocaine metabolites can be found in urine samples for 2 to 4 days (Hadland and Levy, 2016). That is why in the present study, we used two concentrations of each drug to test how the amount of the drug in the sample will affect the action of the adulterant.

Although all urine adulterants have the same general function, which is to conceal the existence of drugs in a urine sample, the method of action for each adulterant may differ. The most common interactions of a urine adulterant with immunoassay tests include binding of the adulterant to drug analytes, modification of the urine sample pH, and increasing the ionic strength of the urine sample. Oxidizing adulterants can interact with antibody proteins or enzymes in the test strip or oxidize drug analytes (Heard and Mendoza, 2007, Luong et al., 2012).

In the current study, vinegar was the most successful adulterant in producing false negative results; it rendered all drug-positive samples negative in both low and high concentrations. This can be explained by the fact that the antigen-antibody interaction during immunoassay drug detection is interrupted by vinegar (Mizrak, 2019). Antigen-antibody complex formation is best when the pH is between 6.5 and 7. The equilibrium constant of the Ag-Ab reaction equation is 100 times lower at pH 5 or 9.5 than it is at

pH 6.5-7, and the antigen-antibody reaction is significantly suppressed on both sides of the maximum (**Riahi-Zanjani, 2014**). Therefore, the interaction between Ag and Ab may not occur and a false negative result may be produced if chemicals introduced to the urine environment can achieve a pH higher than 9.5 or lower than 5. That was confirmed in our study in the results of group I-1, where adding vinegar showed lower pH values in urine.

Our results were in agreement with **Jaffee et al., 2007** who stated that lower pH levels caused by adding vinegar to urine samples can have an impact on binding, times of reaction, and drug solubility. They discovered that vinegar affected the ability to identify THC and amphetamines. **Rajšić et al., 2020** also stated that vinegar turned nearly all UDST results false negative except for cocaine. **Huppertz et al., 2018** found that only acetic acid and vitamin C led to a significant decrease in the measured concentration of the drugs and showed a low pH value.

As regards the bleaching agent, current results revealed that it affected cannabis only. It rendered positive cannabis samples negative in both low and high concentrations. In addition to the dilution effect from adding liquid to a sample, **Chou et al., 2008** ascribed bleach's efficiency as an adulterant to its capacity to degrade analytes and/or deactivate the derivatizing agent through oxidation before and/or during sample preparation. In addition, bleach pH alterations that are more alkaline can influence binding and reaction rates (**Pham et al., 2013**). Bleach was able to change the pH of control sample I-2 to a higher value than normal.

Similar to current results, **Fu, 2016** in his review, included many scientific researches that found decreased immunoassay sensitivity for testing cannabis after adding NaOH bleaching agent. Also, **Pham et al., 2013** stated that a bleaching agent (NaOCl) effectively produced false-negative results across amphetamine samples. **Elsayed et al., 2021** found similar results.

Tetrahydrozoline hydrochloride is the active component in Visine eye drops. It is a decongestant that works by constricting blood vessels to lessen inflammation and redness. However, **Dasgupta, 2007** found that the ability of Visine eye drops to mask the positivity of urine drug samples is mainly attributed to the inert chemicals benzalkonium chloride and borate. In the current work, we found that positive low-concentration cannabis samples turned negative on adding Visine eye drops. This was explained by **Dasgupta, 2010** who stated that THC molecules are promoted to be sequestered into micelle bodies by benzalkonium chloride. This prevents THC from being able to attach to THC-specific antibodies, hence leading to false negative results. Subsequently, increased cannabis concentration will overwhelm the ability of benzalkonium chloride to sequester THC molecules. That is why Visine failed to mask the positivity of the sample in group IIIb-3. Similar results were recorded by **Elkhateeb and Arafa, 2019** and **Elsayed et al., 2021**.

On the other side, the Visine eye drops were discovered by **Rajšić et al., 2020** to be the least effective adulterant in their investigation, which did not alter any of the UDST findings, even for cannabis.

The dilution method is an effective and easy way for adulteration. It can be used in vitro or in vivo; the latter entails the deliberate use of liquids and/or chemicals intended to dilute urine or speed up the body's drug metabolism and/or excretion. Drugs will therefore be present at or close to the cut-off, which may lead to negative results (**Mladěnka et al., 2018**). The in vitro dilution method -as the current work- led to similar results. We found that water was able to turn the positive low-concentration BDZ sample into a negative (group IVa-4) but didn't affect the high-concentration BDZ sample (group IVb-4). Current results agreed with **Elkhateeb and Arafa, 2019** who found that diluted samples were successful in converting positive urine samples into negative. Similarly, according to **Jaffee et al. (2007)**, positive drug tests before consuming fluids revealed a decrease in the concentrations of

marijuana metabolites and cocaine metabolites below cutoff levels enough to produce negative results.

On the other side, **Drummer, 2006** found that the dilution method is not always effective in masking urine drug-positive results, especially in opioid and cocaine samples. He stated that these drugs will cause drug concentrations that are more than 10-fold the cutoff levels, especially when urine is supplied shortly afterward a drug has been used. Due to the practical inability to drink enough water to significantly lower drug concentrations in urine; dilutional adulterants may not have the anticipated negative effect in those people.

CONCLUSION

Immunoassay test strip analysis of abused drugs is a screening test that has many false negative results that may be due to the effect of adulterants. Acidic adulterants such as vinegar are one of the most effective adulterants that succeeded in masking the positivity of drug urine samples in low as well as high concentrations. Bleach comes next, followed by Visine eye drops and water. A positive urine sample that includes drugs may nevertheless be examined and declared "clean and drug-free" after passing a routine screening procedure.

RECOMMENDATIONS

Before doing drug misuse screening tests, we advise testing the physical properties of urine samples to ensure the sample's integrity. Dipstick devices with a wide array of assays in their testing panel can test not only for urine integrity criteria but also for common adulterants. It is vital to develop new drug screening procedures that are more fully resistant to adulterants. Urine should be collected by reputable organizations to protect the subject's privacy and preserve the integrity of the specimen.

STRENGTH OF THE STUDY:

Using two different concentrations of five types of abused drugs is the nearest scenario to real-life situations where

addicts coming to a toxicological lab for testing vary widely in their abuse habits, types of drugs, water drinking habits hence their drug urine concentrations. Drug urine concentration is one of the most important parameters that affect the ability of adulterants to conceal the positivity of samples tested by immunoassay.

LIMITATION OF THE STUDY

The study is better performed with a larger number of adulterants at different concentrations and amounts.

CONFLICT OF INTEREST

All authors declare that there are no conflicts of interest.

REFERENCES

- Abdelati M.A., Hilal M.A., Mohamed K.M., and Elsayed R.M. (2020):** Review on The Effects of Adulterants on Drug Abuse Testing in Urine Samples Ain Shams Journal of Forensic Medicine and Clinical Toxicology, 35: 34-38
- Chou, S.L. Ling, Y.C., Yang, M.H., Giang, Y.S. (2008):** 'Influences of seven Taiwan-produced adulterants on gas chromatographic-mass spectrometric (GC-MS) urinalysis of amphetamines', Journal of the Chinese Chemical Society, 55(3), pp. 682-693
- Dasgupta A. (2010):** Household Chemicals and Internet-Based Products for Beating Urine Drug Tests. Beating drug tests and defending positive results: A toxicologist's perspective.ch 2:61-78
- Dasgupta, A. (2007):** 'The effects of adulterants and selected ingested compounds on drugs-of-abuse testing in urine', American Journal of Clinical Pathology, 128(3), pp. 491-503
- Drummer O.H. (2006):** Drug testing in oral fluid. The Clinical Biochemist. Reviews; 27(3): 147-159.
- Al-khayal R., Al-Mousa F., Attia A., Ragab A., (2017):** Efficiency evaluation of urine collection vessels with impeded urine adulteration/

- substance of abuse (SOA) rapid detection test strips. *Journal of Drug Abuse*. 3 (2): 1-9
- Elkhateeb, S. and Arafa, M. (2019):** 'Influence of zinc and some commercial products on tramadol and apetryl detection in human urine samples', *The Egyptian Journal of Forensic Sciences and Applied Toxicology*, 19(2), pp. 43–64 .
- Elsayed, R.M., Abdel Ati, M. A., Mohamed, K. M., Hilal, M.A. (2021):** Can adulteration of urine samples mask cannabis detection by GC-MS?. *Ain Shams Journal of Forensic Medicine and Clinical Toxicology*, (36), pp. 130–146.
- Fu, S. (2016):** Adulterants in Urine Drug Testing, *Advances in Clinical Chemistry*. 76, pp. 123–163 .
- Hadland, S.E. and Levy, S. (2016):** Objective Testing: Urine and Other Drug Tests. *Child and Adolescent Psychiatric Clinics of North America*, 25(3), pp. 549–565 .
- Heard K. and Mendoza, C.D. (2007):** Consequences of attempts to mask urine drug screens, *Ann. Emerg. Med.* 50 591–592
- Huppertz, B., Bartling, C., & Baum, K. (2018):** Adulteration of Urine Samples, Discovery and Mitigation. *Journal of Applied Life Sciences International*, 16(4), 1–8 .
- Thermo Scientific Indiko Chemistry Analyzer manual, 2013.**
- Jaffee, W.B., Trucco, E., Levy, S., Weiss, R.D. et al., (2007):** Is this urine really negative? A systematic review of tampering methods in urine drug screening and testing. *Journal of Substance Abuse Treatment*, 33(1), pp. 33–42 .
- Karen E.M., Julie C.K., Rabia S.A., Kelly C.L. (2017):** Clinical Interpretation of Urine Drug Tests: What Clinicians Need to Know About Urine Drug Screens, *Mayo Clinic Proceedings*, Volume 92, Issue 5, Pages 774-796.
- Luong, S., Shimmon, R., Hook, J., Fu, S. (2012):** 2-Nitro-6-monoacetylmorphine: potential marker for monitoring the presence of 6-monoacetylmorphine in urine adulterated with potassium nitrite. *Analytical and Bioanalytical Chemistry*, 403, 2057–2063.
- Mizrak, S. (2019):** Fraudulent Methods Causing False Negatives In Urine Drug Testing, *Biomedical Journal of Scientific & Technical Research*, 14(1), pp. 10335–10337 .
- Mladěnka P., Applová, L., Patočka J. (2018):** Comprehensive review of cardiovascular toxicity of drugs and related agents. *Med Res Rev*; 38:1332–1403.
- Olivieri, B.; Marić, M. and Bridge, C. (2018):** Determining the effects of adulterants on drug detection via enzyme-linked immunosorbent assay and adulterant tests strips. *Drug Testing and Analysis*, 10:1383–1393.
- Pham, A.Q.N., Kelly, T. and Fu, S. (2013):** Urine adulteration: can bleach be used to mask MDMA use?. *Anal. Methods*, 5, pp. 3948–3955 .
- Rajšić, I., Javorac, D., Tatović, S., Repić, A., Dukic-Cosić, D., Dordevic, S., et al. (2020):** Effect of urine adulterants on commercial drug abuse screening test strip results. *Arhiv za Higijenu Rada i Toksikologiju*, 71(1), pp. 87–93 .
- Riahi-Zanjani, B. (2014):** False positive and false negative results in urine drug screening tests: Tampering methods and specimen integrity tests. *Pharmacologyonline*, 1, pp. 102–108.

المخلص العربي

تأثير بعض المواد الخادعة على الكشف على مواد الإدمان باستخدام شرائط اختبار المقايسة المناعية

ريهام حسن الفرعوني¹ هبة الله على مبروك² ونجوى محمود حبيب¹

1- قسم الطب الشرعي و السموم الاكلينيكيه- كلية الطب -جامعة المنوفيه
2- قسم الطب الشرعي و السموم الاكلينيكيه- كلية الطب -جامعة كفر الشيخ

المقدمة: يلعب اختبار البول للمخدرات دوراً هاماً في الكشف عن تعاطي المخدرات بصورة مشروعة وغير مشروعة وقد تم توظيف غش عينات البول لتعطيل هذه الاختبارات. تم استخدام العديد من المواد الخادعة بطريقة فعالة في غش عينات البول لتجاوز اختبار المخدرات.

الهدف من البحث: هو تقييم بعض طرق الغش في الكشف عن مواد الإدمان في البول و آثارها على اختبارات الصلاحية.

الطريقة: تم استخدام عينات بول من أشخاص قدموا لإجراء التحليل في معمل قسم الطب الشرعي و السموم الإكلينيكية للكشف عن مواد الإدمان والتي ثبت إيجابيتها لأحد المواد التالية: الترامادول، المورفين، الحشيش، البنزوديازيبين، والأمفيتامين. تم إضافة العديد من المواد الخادعة (الخل، مادة التبييض، قطرة العين فيزيين والماء) وكذلك تخفيف عينة اخرى. تم اختبار هذه العينات لقدرتها على اظهار نتائج سلبية خاطئة في شرائط اختبار المقايسة المناعية.

النتائج: تحليل اختبار المقايسة المناعية للمواد المخدرة هو اختبار فحص يحتوي على العديد من النتائج السلبية الكاذبة التي قد تكون بسبب تأثير المواد الخادعة. هذا و تعتبر المواد الخادعة الحمضية مثل الخل من أكثر المواد فاعلية في إخفاء إيجابية عينات البول من الأدوية بتركيزات مختلفة.