Original article

The Impact of Several Urine Adulterants on Samples that Test Positive for Tetrahydrocannabinol Using Screening Tests

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ABSTRACT

Adulteration, a process that involves manipulating a urine specimen with chemical adulterants to obtain a false negative test result. **Aim:** Assessment of effects of some adulterants on urinary drug testing of tetrahydrocannabinol (THC), comparing effects on two screening methods immunoassay and thin layer chromatography (TLC). **Subjects and methods:** urine samples first were test positive for THC by GC/MS in the toxicology lab at Beni-Suef University. THC positive urine samples were then adulterated by water, HCL bleach, 5% acetic acid, and Tetrahydrozoline eye drops at different concentrations. Finally urine samples were reevaluated for THC using 2 immunoassays, and TLC also, urinary PH, specific gravity, and creatinine and nitrites were assessed. **Results:** The PH of urine significantly decreased after being adulterated with 5% acetic acid at concentrations of 10 and 40, highly significant rise in the PH following 40% Tetrahydrozoline, 10% and 40% HCl bleach and after 300% water dilution. Neither 5% acetic acid nor Tetrahydrozoline at concentration 10 had any impact on urine creatinine levels. HCL-based bleach at concentration 40 and twofold water dilution caused the largest, most significant drop in specific gravity. Except for HCL-based bleach, which included nitrates in 100% of cases at a concentration of 40 and in 76.7% of cases at a concentration of 10, no nitrates were detected in urine samples before the addition of adulterants. Overall accuracy is the best for VIVA E immunoassay except in HCL bleach at 40% and water dilution by 300%, TLC is better. These results showed that VIVA E immunoassay overall accuracy is better than other methods comparing to GC/MS as gold standard for THC before adding adulterants. **Conclusion:** HCl bleach caused the most remarkable changed to urine parameters. VIVA E immunoassay overall accuracy is better than other methods comparing to GC/MS.

Keywords: Adulteration; Tetrahydrocannabinol; Accurate Card; autoanalyzer, HCL-based bleach

I-INTRODUCTION

Many governments have drug testing systems in place to help create drug-free workplaces, especially in fields where safety is a concern, such the military, transportation, mining, and any other industry that uses heavy machinery. Aside from helping law enforcement authorities, drug testing systems are also set up to help with clinical intoxication treatment, harm reduction efforts, and rehabilitation programmes (Smith et al., 2021).

Blood, urine, and oral fluid are just a few of the several biological matrices that can be employed for drug testing applications (Morato et al., 2019).

The most common matrix among the different alternatives available to toxicologists is urine. For a number of reasons, including the ease and non-intrusiveness of its gathering, this matrix is regarded as the most ideal. Additionally, the majority of drugs of interest and/or their metabolites can be detected in urine over a reasonably long period of time (Wagmann et al., 2022).

Urine samples are occasionally intentionally corruptive by intake or addition of a foreign contaminants in order to hinder the identification of illegal substances. Tampering techniques often use dilution with water and addition of foreign chemicals e.g sodium hypochlorite bleach, acetic acid, visine eye drops, water plus diuretics (Chen et al., 2014).

Due to this kind of drug testing's popularity, numerous regulations and executions have been created over the past three decades or more to regulate it, and they have been modified to be appropriate in both clinical and medico-legal frames (Fu, 2019).

An initial screening test and a subsidiary confirmatory test are frequently used in urine drug testing. Typically, immunoassay tests are used for the first screening procedures. Any specimen that shows a positive result in the screening tests is subsequently put through asserted testing employing liquid or gas chromatography-mass spectrometry (GC MS) (LC MS) (Fu et al., 2014).

Clinical toxicology and drug check in biological matrices both frequently use immunoassay-based drug screening techniques. Automated systems carry out immunoassay tests. Each instrument can be fitted with a variety of xenobiotics-covering kits from various manufacturers. These

techniques don't require sample preparation, are simple to use, and offer quick results for each drug class in support of medical treatment, occupational drug check, emergency toxicology, detoxification, and remedy. The immunoassay is a biochemical technique that uses an antigen-antibody interaction to identify xenobiotics (Graziano et al., 2019).

We sought to compare the impact of commercially available easy obtained adulterants on 2 different screening methodologies Accurate Card VIVA E immunoassay auto analyzer and TLC for THC urine drug tests utilizing various adulteration techniques.

II-SUBJECTS and METHOD

Previously coded urine samples were obtained from the Faculty of Medicine, Beni-Suef University, Egypt's forensic and toxicological lab from reservoir samples with no participant names on. There were 62 urine samples in total. From the beginning of January 2022 until the end of December 2022, samples were taken. Samples were divided into two categories: group -32 THC Negative by GC/MS served as no adulteration control group; 30 samples THC Positive by GC/MS after immunoassay screening served as adulteration group.

Samples from adulteration group were divided in to; a sample before adulteration, samples after the addition of 10%, 40% concentrations of 5% acetic acid, 10%, 40% concentrations of bleach, 10%, 40% concentrations of Tetrahydrozolinee eye drops, and a sample after dilution by 100% and 300% of water were all distributed among nine cups.

Standard solutions of THC, Δ9-THC, and cannabinol (CBN) were secured by Sigma-Aldrich Chimie (Saint Quentin Fallavier, France). Hexanes, diethyl ether, Methanol, toluene, acetonitrile, and dichloromethane solvents were secured by Sigma-Aldrich Chimie (Saint Quentin Fallavier, France). 10- × 20-cm plates (precoated silicagel HPTLC F254) (Merck Art. 11764) (VWR International SAS, Fonterlaysous Bois, France), Readymade Fast Blue B reagent, THC urine HEIA 100 ml kit and PH 0—14 paper, were purchased from sigma-Aldrich Egypt.

Urine analysis strips purchased from SGL**.** medi test Germany company. Adulterants**;** household 5% acetic acid,

Tetrahydrozolinee eye drops, Hypochlorite-Based bleach all purchased from local markets.

The used Gas chromatography mass spectrometry (GC-MS) was scientific Trace 1300 equipped from THERMO Company. The used Autoanalyzer was VIVA E immunoassay equipped from SEIMENS Company. The used Biochemistry Analyzer was Semi – Automated Chemistry Analyzer Robonik Prietest Touch (Indian).

Each sample was divided to 9 cups: sample before adulteration, sample after adding 10 %, 40 % 5% acetic acid, 10 %, 40 % bleach, 10 %, 40 % Tetrahydrozolinee eye drops and samples after water dilution by 100 % & 300 %. Specimen collection, storage and urine preparation were according to Hawks & Chiang, 1986.

3 mL of a solvent admixture of acetonitrile: dichloromethane (1:3) were positioned in test tube for extraction. Aliquot of 5mL of prepared urine sample was added and rattle gently for about 1 minute manually.

Blending was continued on a stir on the roller for ten minutes, and lastly centrifuged for ten minutes at 3000 rpm. The top organic stratum was split up into a vial and evaporated to drought under a stream of nitrogen flow at average temperature of the room. The derivatization was held with 30 µL MSTFA +1% TMCS, vortexed for five seconds and pliable to stand at 25ºC for 30 min to fulfill silylation. The extract was moved to 250 µL vial. An aliquot of 1 µL of the all set extract was injected into the GC-MS device (Vidic, 2020).

The HP-5MS 15-m 0.25-mm 0.25-m capillary column was utilized, along with a helium (99.99%) carrier gas at a flow rate of 1.3 mL min-1. The injector temperature was kept at 250 ºC, and all injections were performed in splitless mode. The GC oven temperature was kept at 50 ºC for 1 minute before being set to 250 ºC at 10 ºC min-1 and held for 10 minutes. The GC-MS transfer line was preserved at 280 ºC, electron ionization at 70eV, and the mass spectrum was recorded (Galand et al., 2004), the intensity was shown as mass-to-charge ratio in the mass spectrum. The base peak is the peak with the greatest intensity in the THC spectrum.

The intensity of the base peak is normalized to produce relative intensities. THC in urine GC/MS tests

use fused silica capillary columns with methyl silicone or 5% phenylmethylsilicone stationary phases. Electron ionization (EI) is still the most used technique for ionizing derivatized THC as shown in (Figure 1). Each of the described THC derivatives provides at least three ions with high relative intensities using EI-MS (Tsai, 2007).

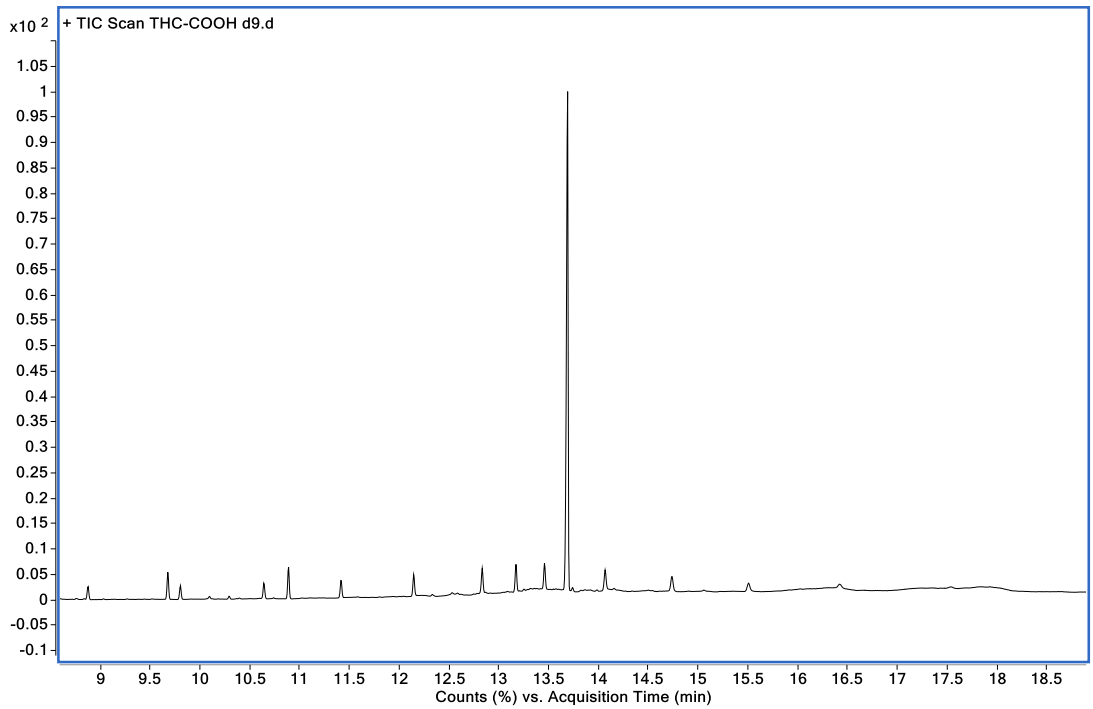

Figure 1: GC/MS chromatogram of d9-THC-COOH positive sample (scan mode)

Adulterants were admixed to drug-positive urine samples (1 mL) in various volumes to simulate lifetime actions in which common population do not have the ability to accurately quantify the exact quantity but attempt not to alter the ordinary look of the urine to avoid getting caught cheating.

Following manufacturer directions, urine samples containing the masking agents and the control urine were analyzed for signs of THC masking using the URIT 11G urine reagent bands (Medical Electronic Co. Ltd., Guangxi, China). We also utilized pH test strips (Macherey-Nagel GmbH & Co. KG, Düren, Germany) with a pH range of 0-14. PH, specific gravity, creatinine, nitrate, blood, protein and glucose are used to determine certain urine characters which help in detection of adulterated urine, and to detect cut off to detect adulterants with each method using urine analysis strips as shown in (Figure 2).

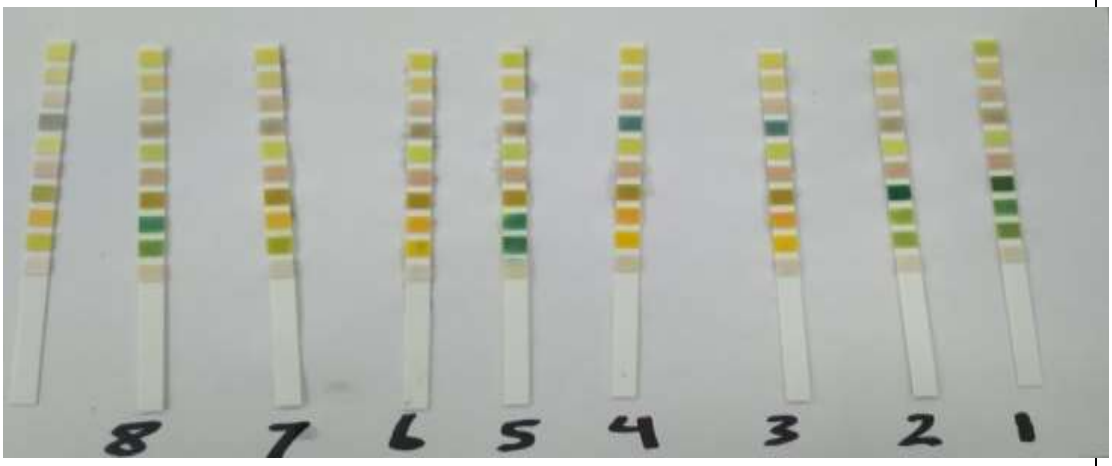

Figure 2: urine reagent bands testing before and after adulteration

No number strip: negative control strip before use. *Strip 1*: after adding HCl bleach 10 %, , *Strip 2*: after adding HCl bleach 40 %, *Strip 3*: after adding 5% acetic acid at 10 % , *Strip 4*: after adding 5% acetic acid at 40 %, *Strip 5*: after adding Tetrahydrozolinee eye drops , *Strip 6*: after water dilution by 100 %, *Strip 7*: after water dilution by 300 %, *Strip 8*: control urine with no adulterants.

Preliminary urine testing for THC after adulteration by Autoanalyzer immunoassay; VIVA E immunoassay. kits were used exactly as directed by the manufacturer. Two droplets of urine were sucked into the test sample site. Then, the outcomes expressed in numbers on result sheet, Cut-off point for THC is 50 ng/mL. The Autoanalyzer assay THC Kits contains G6PDH labeled THC in 100 ml tris buffer. The reduction of the cofactor nicotinamide adenine dinucleotide (NAD) to NADH is combined with the oxidation of the enzyme substrate G6P to create glucuronolactone-6-phosphate. When there are no pharmaceuticals present in the specimen, the antibodies attach to the enzyme-labeled medicines and suppress their enzymatic vigor. Unconjugated THC in the material contests for antibody binding; resulting in little number of antibodies obtainable for binding to rG6PDH- THC conjugates and reduced inhibition of rG6PDH activity. The rate of NADH generation, as measured by the change in absorbance at 340 nm, is proportional to the activity of the G6PDH enzyme. As a result, the absorbance alteration was plotted against the THC calibrator concentration to create a calibration curve for executing a semi-quantitative test (Tsai, 2007).

Preliminary urine testing after adulteration by rapid immunoassay diagnostic test (Acurrate card) for THC positive or negative samples, All UDST kits were used exactly as directed by the manufacturer. Test strip were put on 5 ml of urine. After 3-5 minutes, the findings were interpreted. Because these investigations are qualitative, a positive result indicates a substance concentration greater than the cut-off level. Cut-off point for THC is 50 ng/mL, as shown in (Figure 3).

A membrane-based, dry chemistry, single phase lateral-flow immunochromatography was used. The 3001JL urine sample fluxes from the barrage to nitrocellulose band through a sample pad. The nitrocellulose strips are impregnated with reagents that use competitive immunoassay to reveal the existence of THC or its metabolites.

The reagent strips comprise antibody-coated, red-dyed microparticles that become pendent in the urine sample and move down the strip with the urine specimen to a detection district containing congealed drug conjugate. If the target drug is existent in significant concentrations in the urine specimen, it will fasten with the antibody on the microparticles, preventing it from fasten with the drug conjugate at the detection zone (Towt et al., 1995).

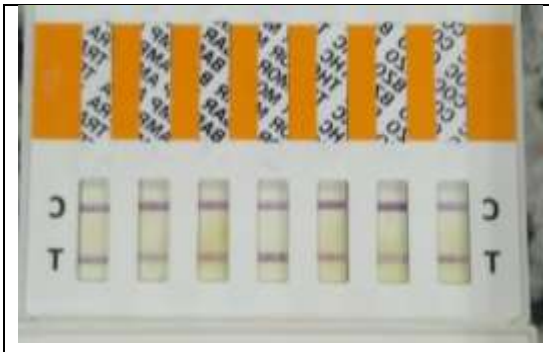

Figure 3a: Accurate Card immunoassay for THC negative samples after adding adulterants

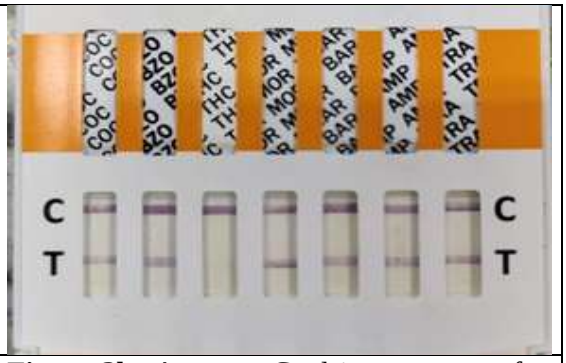

Figure 3b: Accurate Card immunoassay for THC positive sample before adding adulterants

Preliminary urine testing after adulteration by TLC analysis for THC. TLC employs capillary forces to transport the mobile phase hexane–diethyl ether (80:20, v/v) through the layer of a solid phase consists of 10- × 20-cm sheets (precoated silicagel HPTLC F254), Readymade Fast Blue B reagent was used as visualizing reagent

(Galand et al., 2004) ,as shown in (Figure 4).

Ethical considerations:

Urine Samples were analyzed as secondary data analysis; so informed consent from cases is not applied. Ethical approval was obtained from the ethical committee of scientific research, Faculty of Medicine, Beni-Suef University, approval number (FMBSUREC/05032023/ Abdelaziz).

Statistical analysis:

The data were analyzed using SPSS version 25; the scale variables were described using the terms mean, standard deviation (SD) as they were normally distributed. We presented the categorical variables as numbers (No.), and percentages (%). The scale variables were compared between the various adulterant groups and the control group using the ANOVA test. Then we used Tukey Post-hoc test to assess the differences between each 2 groups. Chi-Squared test was used to assess the differences in the detection of nitrates appearance caused by adulteration using 5% acetic acid, Tetrahydrozoline, HCl bleach and water to the urine samples during drug examination.

Specificity (SP), sensitivity (SN), Positive predictive value (PPV) and negative predictive value (NPV) were calculated using cross tabs and bases. P-values 0.05 indicate significant values.

III-RESULTS

This study conducted on samples collected from forensic and toxicological Beni-Suef lab (forensic and clinical toxicology department, Faculty of Medicine, Beni-Suef University).Total number of cases was 62.

Table (1) shows that adding 5% acetic acid at 10 and 40 concentrations caused a highly statistically significant decrease in the pH, adding Tetrahydrozoline at 10 concentrations caused a significant increase in the pH, adding Tetrahydrozoline at 40 concentrations caused a highly significant increase in the pH, adding 10 and 40 concentrations of HCl bleach caused a significant increase in the pH, and adding water at double the concentration caused the greatest increase in the pH.

Table (2) shows that 5% acetic acid or Tetrahydrozoline at a concentration of 10 had no impact on the urine creatinine level, while the addition of HCl bleach at a concentration of 10 or

water dramatically reduced the creatinine level. There was a greater reduction in creatinine at concentration 40 when using 5% acetic acid, Tetrahydrozoline, HCl bleach, and water dilution; the highest significance was seen with double water dilution, followed by HCl bleach. Although HCl bleach's creatinine level considerably fell, it was still over the acceptable range of 20 mg/dl.

Table (3) shows that the maximum significant decrease in the specific gravity was noticed in HCl bleach at concentration 40 and double dilution of water (P-value<0.001). Although, non-significant specific gravity change was noticed on Tetrahydrozoline addition in both concentrations; but adding 5% acetic acid at 10 and 40 concentrations caused high statistically significant increase (P-value<0.001).

Table (4) shows that except for HCl bleach, where nitrates were found in 100% of instances at a 40 concentration and in 76.7% of cases at a 10 concentration (P-value 0.001), there were no nitrates found in the urine samples before the addition of the adulterants at any concentration or dilution.

The other part of this study is comparing TLC, accurate card and VIVA E auto-analyzer immunoassay, that was done through validation of all of them and measuring SP, SN, PPV and NPV before and after adding adulterants comparing to GC/MS as gold standard for THC.

Table (5) shows that regarding the validation of the Accurate Card before adding adulterants compared to GC/MS as a gold standard for THC detection, the study showed NPV was 89.7%, SP was 86.7%, PPV was 87.9% and SN was 90.6% for THC. Regarding the validation of VIVA E analyzer immunoassay before adding adulterants compared to GC/MS, the study showed NPV was 100 %, SP was 100%, PPV was 97.1% and SN was 96.6% for THC. Regarding the validation of TLC before adding adulterants compared to GC/MS, the study showed NPV was 93.9%, SP was 93.3%, PPV was 93.1% and SN was 93.1% for THC. These results showed that VIVA E immunoassay overall accuracy is better than other methods comparing to GC/MS as gold standard for THC before adding adulterants. The overall accuracy was the best for VIVA E immunoassay before adulteration, after adding 5% citric acid at 10% and 40%

dilution, and Tetrahydrozoline eye drops at 10% and 40% dilution. The overall accuracy was the best for TLC after adding HCL bleach at 10% and 40% dilution and after dilution with water by 100% and 300%.; so overall accuracy is the best for VIVA E immunoassay except in HCL bleach at 40% and water dilution by 300%, TLC is better.

In table (6) and figure (5): the overall accuracy was the best for VIVA E immunoassay before adulteration, after adding vinegar 10%, vinegar 40%, visin 10% and 40%. While the overall accuracy was the best for TLC after adding Clorox 10% and 40%, after dilution 100% and 300%.

Table 1: The effect of adulteration by 5% acetic acid, Tetrahydrozoline eye drops, HCl bleach and water on the pH of the urine samples during drug examination:

Adulterant		PH of urine sample				P-value
		5% acetic acid	TETRAHYDROZOLINE	HCl bleach	Dilution (H_2O)	
A.Control	Mean±SD	5.621±0.6162				<0.001* One-way ANOVA
B.Adulterant at 10 conc/dil	Mean±SD	3.97±0.45	5.79±0.49	7.48±0.92	6.09±0.91	
C.Adulterant at 40 conc/dil	Mean±SD	3.53±0.43	6.09±0.4	9.3±0.8	6.29±0.44	
Tukey Post-hoc pairwise comparison						
P-value (A&B)		<0.001**	0.046*	<0.001**	<0.001**	
P-value (A&C)		<0.001**	<0.001**	<0.001**	<0.001**	
P-value (B&C)		<0.001**	<0.001**	<0.001**	0.097	

Data was presented as mean±SD *p-value is significant at <0.05, **p-value is significant at <0.001(highly significant) HCL: hydrochloric acid

Table 2: The effect of adulteration by 5% acetic acid, Tetrahydrozoline, HCl bleach and water on the creatinine of the urine samples during drug examination:

Adulterant		Creatinine of urine sample				P-value
		5% acetic acid	Tetrahydrozoline	HCl bleach	Dilution (H_2O)	
A.Control	Mean±SD	61.8±22				<0.001* One-way ANOVA
B.Adulterant at 10 conc/dil	Mean±SD	55.8±20.7	55.8±20.7	44.1±18	31.2±10.9	
C.Adulterant at 40 conc/dil	Mean±SD	46.8±17.8	46.8±19.1	32.8±15.5	15.9±5.9	
Tukey Post-hoc pairwise comparison						
P-value (A&B)		0.085	0.097	<0.001**	<0.001**	
P-value (A&C)		<0.001**	<0.001**	<0.001**	<0.001**	
P-value (B&C)		0.005*	0.004*	<0.001**	<0.001**	

Data was presented as mean±SD *p-value is significant at <0.05 , **p-value is significant at <0.001(highly significant) HCL: hydrochloric acid

Table 3: The effect of adulteration by 5% acetic acid, Tetrahydrozoline, HCl bleach and water on the specific gravity of the urine samples during drug examination:

Adulterant		Specific gravity of urine sample				P-value
		5% acetic acid	Tetrahydrozoline	HCl bleach	Dilution (H_2O)	
A.Control	Mean±SD	1023.2±5.5				One-way ANOVA
B.Adulterant at 10 conc/dil	Mean±SD	1050±5	1025±6.26	1018±4.6	1019±6.08416	
C.Adulterant at 40 conc/dil	Mean±SD	1050±5	1021±5.3	1006±5.7	1007±4.00837	
Tukey Post-hoc pairwise comparison						
P-value (A&B)		<0.001**	0.075	<0.001**	<0.001**	
P-value (A&C)		<0.001**	0.025*	<0.001**	<0.001**	
P-value (B&C)		1.000	<0.001**	<0.001**	<0.001**	

Data was presented as mean±SD *p-value is significant at <0.05, **p-value is significant at <0.001(highly significant)

Table 4: Detection of nitrates appearance caused by adulteration using 5% acetic acid, Tetrahydrozoline, HCl bleach and water to the urine samples during drug examination

Adulterant		Nitrate of urine sample no.(%)			
		5% acetic acid	Tetrahydro zoline	HCl bleach	Dilution (H_2O)
A.Control	Positive Negative	0 (0) 100 mg/ dL(100%)			
B.Adulterant at 10 conc/dil	Positive Negative	0(0) 100(100%)	0(0) 100(100%)	79(76.7%) 24(23.3%)	0(0) 100(100%)
C.Adulterant at 40 conc/dil	Positive Negative	0(0) 100(100%)	0(0) 100(100%)	100(100%) 0(0)	0(0) 100(100%)
P-value (A&B) x^2 test		-------	-------	<0.001**	-------
P-value (A&C) x^2 test		-------	-------	<0.001**	-------
P-value (B&C) x^2 test		--------	--------	0.002*	--------

Data was presented as number and percent *P-value is significant at <0.05 , **P-value is significant at <0.001(highly significant), X^2: Chi-Squared test

Table 5: Validation of (Accurate card – VIVA E immunoassay- TLC) before and after adding adulterants comparing to GC/MS as gold standard for THC

Adulterant	Analysis method	NPV	SP	PPV	SN	Accuracy
Before	CARD	90.6%	87.9%	86.7%	89.7%	90.2%
	AUTO	97.1%	100%	100%	96.6%	98.4%
	TLC	93.9%	93.3%	93.1%	93.1%	93.5%
5% acetic acid by 10% dilution	CARD	73.2%	90.9%	85.7%	62.1%	77.4%
	AUTO	94.3%	100%	100%	93.1%	96.8%
	TLC	91.2%	93.3%	92.9%	89.7%	91.9%
5% acetic acid 40% dilution	CARD	56.6%	90.9%	66.7%	20.7%	58.1%
	AUTO	86.5%	97%	96%	82.8%	96.4%
	TLC	82.1%	97%	95.7%	75.9%	87.1%
Tetrahydrozoline 10% dilution	CARD	76.3%	87.9%	83.3%	69%	79%
	AUTO	100%	97%	96.7%	100%	98.4%
	TLC	96.9%	93.9%	93.3%	96.6%	95.2%
Tetrahydrozoline 40% dilution	CARD	76.3%	87.9%	83.3%	69%	79%
	AUTO	94.1%	97%	96.4%	93.1%	95.2%
	TLC	86.1%	93.9%	92.3%	82.8%	88.7%
Hcl bleach by 10% dilution	CARD	51.7%	93.9%	0%	0%	50%
	AUTO	78.6%	100%	100%	69%	85.5%
	TLC	82.1%	97%	95.7%	75.9%	87.1%
Hcl bleach by 40% dilution	CARD	52.6%	90.9%	40%	6.9%	51.6%
	AUTO	56.9%	100%	100%	13.8%	59.7%
	TLC	62.7%	97%	90.9%	34.5%	67.7%
Water Dilution 100%	CARD	76.9%	90%	87%	69%	80.6%
	AUTO	91.7%	100%	100%	89.7%	96.7%
	TLC	94.1%	97%	96.4%	93.1%	95.2%
Water Dilution 300%	CARD	54.2%	97%	66.7%	6.9%	54.8%
	AUTO	89.2%	100%	100%	86.2%	93.5%
	TLC	91.7%	100%	100%	89.7%	95.2%

Specificity (SP), sensitivity (SN), Positive predictive value (PPV), negative predictive value (NPV), *P-value is significant at <0.05 , **P-value is significant at <0.001(highly significant)
THC : tetrahydrocannabinol TLC: thin layer chromatography GC/MS: gas-chromatography mass/spectrometry

Sensitivity, specificity, positive predictive value, and negative predictive value was calculated based on the true positive, true negative, false positive, false negative results of the comparison between Immunoassays and TLC compared to the reference GC/MS

Table 6: THC results of accurate card – VIVA E immunoassay- TLC before and after adding adulterants

analysis method			GC mass		Total
			Negative	Positive	
CARD before adulterant	Negative	Number	29	3	32
		% card (row total)	90.6%NPP	9.4%	100.0%
		% GC (column total)	87.9%SP	10.3%	51.6%
	Positive	Number	4	26	30
		% card (row total)	13.3%	86.7%PPV	100.0%
		% GC (column total)	12.1%	89.7% SN	48.4%
VIVA E analyzer immunoassay before adulterant	Negative	Number	33	1	34
		% VIVA E ANALYZER (row total)	97.1%	2.9%	100.0%
		% GC (column total)	100.0%SP	3.4%	54.8%
	Positive	Number	0	28	28
		% VIVA E ANALYZER (row total)	0.0%	100.0%PPV	100.0%
		% GC (column total)	0.0%	96.6%SN	45.2%
TLC before adulteration	Negative	Number	31	2	33
		%TLC (row total)	93.9%NPV	6.1%	100%
		%GC (column total)	93.9% SP	6.9%	53.2%
	Positive	Number	2	27	29
		%TLC (row total)	6.9%	93.1%PPV	100%
		%GC (column total)	6.1%	93.1% SN	46.8%
CARD after 10% Vinegar	Negative	Number	30	11	41
		% CARD (row total)	73.2%NPV	26.8%	100.0%
		% GC (column total)	90.9% SP	37.9%	66.1%
	Positive	Number	3	18	21
		% CARD (row total)	14.3%	85.7%PPV	100.0%
		% GC (column total)	9.1%	62.1% SN	33.9%
	Negative	Number	33	2	35

Viva E analyzer immunoassay after 10% Vinegar		%Viva E analyzer(row total)	94.3%NPP	5.7%	100.0%
		% GC (column total)	100.0%SP	6.9%	56.5%
	Positive	Number	0	27	27
		%Viva E analyzer(row total)	0.0%	100%PPV	100.0%
		% within GC mass	0.0%	93.1%SN	43.5%
TLC after 10% Vinegar	Negative	Number	31	3	34
		%TLC(row total)	91.2%NPV	8.8%	100.0%
		% GC(column total)	93.9%SP	10.3%	54.8%
	Positive	Number	2	26	28
		%TLC(row total)	7.1%	92.9% PPV	100.0%
		% GC(column total)	6.1%	89.7% SN	45.2%
card after 40% vinegar	Negative	Number	30	23	53
		% card(row total)	56.6%NPV	43.4%	100.0%
		% GC (column total)	90.9%SP	79.3%	85.5%
	Positive	Number	3	6	9
		% card(row total)	33.3%	66.7%PPV	100.0%
		% GC (column total)	9.1%	20.7%SN	14.5%
Viva E analyzer immunoassay after vinegar 40%	Negative	Number	32	5	37
		% viva E (row total)	86.5%NPV	13.5%	100.0%
		% GC (column total)	97.0%SP	17.2%	59.7%
	Positive	Number	1	24	25
		% viva E (row total)	4.0%	96.0%PPV	100.0%
		% GC (column total)	3.0%	82.8%SN	40.3%
TLC after vinegar 40%	Negative	Number	32	7	39
		% TLC (row total)	82.1%NPV	17.9%	100.0%
		%GC(column total)	97.0%SP	24.1%	62.9%
	Positive	Number	1	22	23
		% TLC (row total)	4.3%	95.7%PPV	100.0%
		%GC(column total)	3.0%	75.9%SN	37.1%
CARD after 10% visin	Negative	Number	29	9	38
		% CARD(row total)	76.3%NPV	23.7%	100.0%
		% GC(column total)	87.9%SP	31.0%	61.3%
	Positive	Number	4	20	24
		% CARD(row total)	16.7%	83.3%PPV	100.0%

		% GC(column total)	12.1%	69.0%SN	38.7%
Viva E analyzer immunoassay after visin 10 %	Negative	Number	32	0	32
		%Viva E analyzer (row total)	100%NPV	0.0%	100%
		%GC(column total)	97.0%SP	0.0%	51.6%
	Positive	Number	1	29	30
		%Viva E analyzer (row total)	3.3%	96.7%PPV	100%
		%GC(column total)	3.0%	100% SN	48.4%
TLC after 10% visin	Negative	Number	31	1	32
		% TLC(row total)	96.9%NPV	3.1%	100.0%
		% GC(column total)	93.9%SP	3.4%	51.6%
	Positive	Number	2	28	30
		% TLC(row total)	6.7%	93.3%PPV	100.0%
		% GC(column total)	6.1%	96.6%SN	48.4%
CARD after 40% visin	Negative	Number	29	9	38
		% CARD (row total)	76.3%NPV	23.7%	100.0%
		%GC (column total)	87.9%SP	31.0%	61.3%
	Positive	Number	4	20	24
		% CARD (row total)	16.7%	83.3%PPV	100.0%
		%GC (column total)	12.1%	69.0%SN	38.7%
Viva E analyzer immunoassay after 40% visin	Negative	Number)	32	2	34
		% Viva E analyzer (row total)	94.1%NPV	5.9%	100.0%
		%GC(column total)	97.0%SP	6.9%	54.8%
	Positive	Number)	1	27	28
		% Viva E analyzer (row total)	3.6%	96.4%PPV	100.0%
		%GC(column total)	3%	93.1%SN	45.27%
TLC after 40 % visin	Negative	Number	31	5	36
		TLC (row total)	86.1%NPV	13.9%	100.0%
		% GC (column total)	93.9%SP	17.2%	58.1%
	Positive	Number	2	24	26
		TLC (row total)	7.7%	92.3%PPV	100.0%
		% GC (column total)	6%	82.8%SN	38.7%
	Negative	Number	31	29	60

CARD after 10% Clorox		% CARD (row total)	51.7%NPV	48.3%	100.0%
		% GC(column total)	93.9%SP	100.0%	96.8%
	Positive	Number	2	0	2
		% CARD (row total)	100.0%	0.0%PPV	100.0%
		% GC(column total)	6.1%	0.0%SN	3.2%
Viva E analyzer immunoassay after 10% Clorox	Negative	Number	33	9	42
		%Viva E analyzer (row total)	78.6%NPv	21.4%	100.0%
		%GC(column total)	100.0%SP	31.0%	67.7%
	Positive	Number	0	20	20
		%Viva E analyzer (row total)	0.0%	100. %PPV	100.0%
		%GC(column total)	0.0%	69.0%SN	32.3%
TLC after clorox 10%	Negative	Number	32	7	39
		% TLC(row total)	82.1%NPV	17.9%	100.0%
		% GC(column total)	97.0%SP	24.1%	62.9%
	Positive	Number	1	22	23
		% TLC(row total)	4.3%	95.7%PPV	100.0%
		% GC(column total)	3.0%	75.9% SN	37.1%
CARD after 40% Clorox	Negative	Number	30	27	57
		% CARD (row total)	52.6%NPV	47.4%	100.0%
		% GC (column total)	90.9%SP	93.1%	91.9%
	Positive	Number	3	2	5
		% CARD (row total)	60.0%	40.0%PPV	100.0%
		% GC (column total)	9.1%	6.9%SN	8.1%
Viva E analyzer immunoassay after 40% Clorox	Negative	Number	33	25	58
		%Viva E analyzer (row total)	56.9%NPV	43.1%	100.0%
		% within GC(column total)	100.0%SP	86.2%	93.5%
	Positive	Number	0	4	4
		%Viva E analyzer (row total)	0.0%	100.0%PPV	100.0%
		% within GC(column total)	0.0%	13.8%SN	6.5%
	Negative	Number	32	19	51

TLC after 40% Clorox		%TLC (row total)	62.7%NP	37.3%	100.0%
		% GC(column total)	97.0%SP	65.5%	82.3%
	Positive	Number	1	10	11
		%TLC (row total)	9.1%	90.9% PPV	100.0%
		% GC(column total)	3.0%	34.5%SN	17.7%
CARD after 100% water	Negative	Number	30	9	39
		% CARD (row total	76.9%NPV	23.1%	100.0%
		% GC (column total)	90.9%SP	31.0%	62.9%
	Positive	Number	3	20	23
		% CARD (row total	13.0%	87.0%PPV	100.0%
		% GC (column total)	9.1%	69.0%SN	37.1%
Viva E analyzer immunoassay after 100% water	Negative	Number	33	3	36
		% Viva E analyzer(row total)	91.7%NPv	8.3%	100.0%
		% GC (column total)	100.0%SP	10.3%	58.1%
	Positive	Number	0	26	26
		% Viva E analyzer(row total)	0.0%	100.0%PPV	100.0%
		% GC (column total)	0.0%	89.7%SN	41.9%
TLC after 100% water	Negative	Number	32	2	34
		% TLC(row total)	94.1%NPV	5.9%	100.0%
		% GC (column total)	97.0%SP	6.9%	54.8%
	Positive	Number	1	27	28
		% TLC(row total)	3.6%	96.4%PPV	100.0%
		% GC (column total)	3.0%	93.1%SN	45.2%
CARD after 300% water	Negative	Number	32	27	59
		CARD (row total)	54.2%NPV	45.8%	100.0%
		% within GC(column total)	97.0%SP	93.1%	95.2%
	Positive	Number	1	2	3
		CARD (row total)	33.3%	66.7%PPV	100.0%
		% within GC(column total)	3.0%	6.9%SN	4.8%
	Negative	Number	33	4	37

Viva E analyzer immunoassay after 300% water		% Viva E analyzer (row total)	89.2%NPV	10.8%	100.0%
		% GC(column total)	100.0%SP	13.8%	59.7%
	Positive	Number	0	25	25
		% Viva E analyzer (row total)	0.0%	100.0%PPV	100.0%
		% GC(column total)	0.0%	86.2%SN	40.3%
TLC after 300% water	Negative	Number	33	3	36
		% TLC (row column)	91.7%NPV	8.3%	100.0%
		% GC (column total)	100.0%SP	10.3%	58.1%
	Positive	Number	0	26	26
		% TLC (row column)	0.0%	100.0%PPV	100.0%
		% GC (column total)	0.0%	89.7%SN	41.9%

Sensitivity, specificity, positive predictive value, and negative predictive value was calculated based on the true positive, true negative, false positive, false negative results of the comparison between the tested ----- compared to the reference GC ----

Figure 4a: TLC for THC, A. THC standard, B. after adulteration with 5% citric acid C. after adulteration with visin eye drops from left to right. Silica gel plates spraying with acidified Fast Blue

Figure 4b: TLC for THC before adulteration of THC positive sample, D. after adding Clorox, E. after water dilution, F. THC standard from right to left. Silica gel plates spraying with acidified Fast Blue

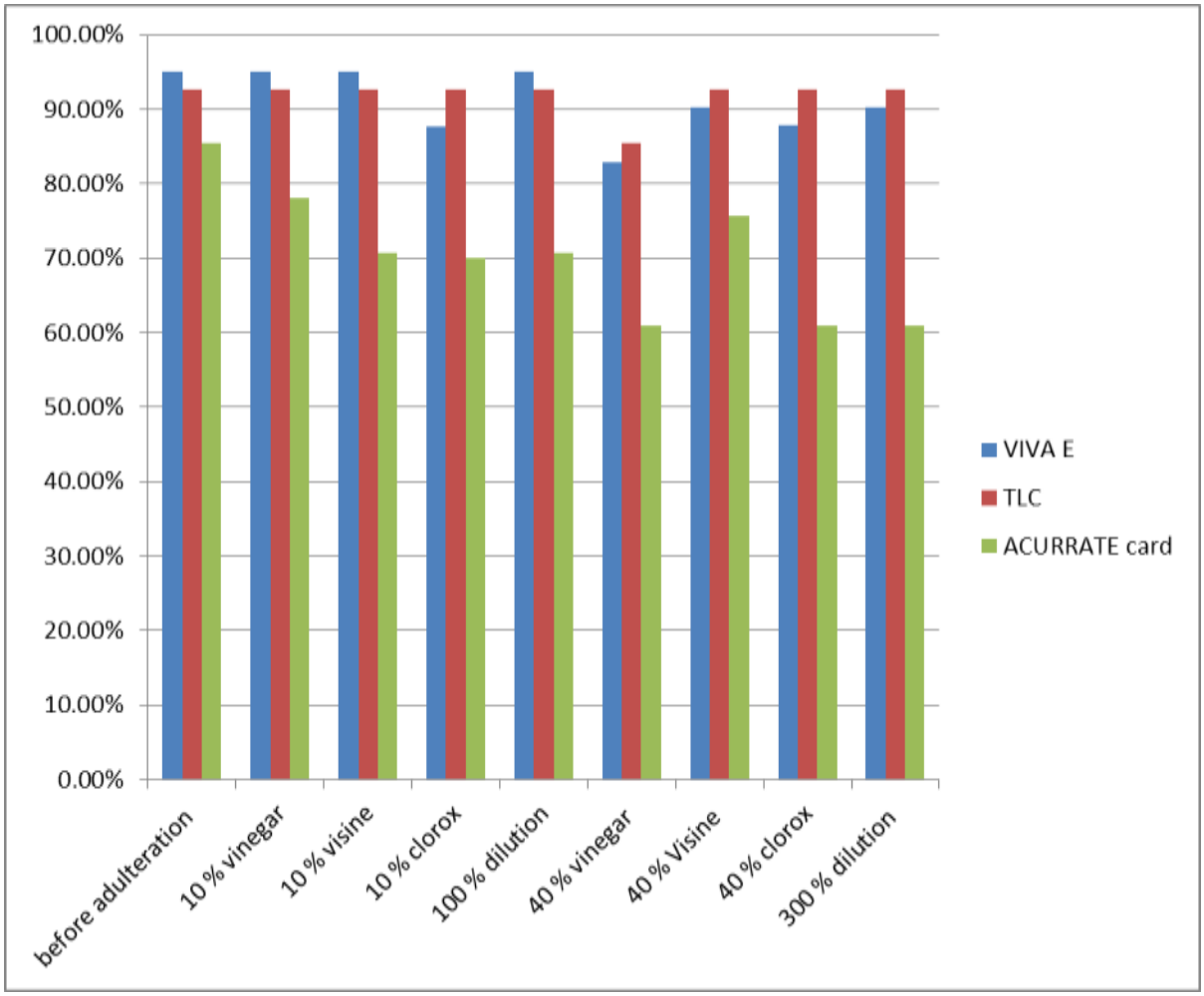

Figure 5: Accuracy comparing between (VIVA E immunoassay, TLC and Accurate card) before adulteration and after adding different adulterant in THC samples.

IV-DISCUSSION:

Because they are seen to pose unacceptably high risks of addiction to consumers, illicit substances are those for which non-medical use has been outlawed by international drug control treaties for fifty years. Since then, the international regulation of drugs has expanded beyond those derived from plants, such as heroin, cocaine, and cannabis, to include synthetic substances like amphetamines and methylene dioxymetamfetamine (MDMA), as well as medicines like buprenorphine, methadone, and benzodiazepines (Degenhardt and Hall, 2012).

Addictive drug abusers commonly employ various methodologies (eg, adulteration, urine substitution, diluting urine) to escape detection. A requisite understanding of urine figures is helpful when interpreting drug screen results.

Average urine color ranges from pale yellow to lucid. The temperature of the specimen should be listed within four minutes following collection and is commonly between 32.2°C and 37.8°C. Urine temperature may hold at 32.5°C for up to fifteen minutes. Urinary pH swages between 4.5 and 8. Specific gravity commonly swages between 1.002 and 1.030. Creatinine concentrations should exceed 20 mg/dL in urine of healthy individuals (Moeller, 2017).

The point of the running investigation was to show how various commercially available easy obtained adulterants, affect THC drug tests in the urine, Contrasting the impacts of modern techniques of their detection through the effects on (pH, Creatinine, Specific gravity and Nitrite) of urine samples on screening methods (TLC, Accurate Card, and VIVA E auto analyzer immunoassay). In the current work; the PH of urine significantly decreased after being adulterated with 5% acetic acid at concentrations of 10 and 40. Contrarily, there was a highly significant rise in the PH following the addition of 40% Tetrahydrozoline and a considerable increase following the addition of 10% and 40% HCl bleach, respectively. After dilution with water, a twofold dilution of water was required to achieve the maximal PH rise.

A specimen is considered legitimate (i.e., authentic urine), according to Kim et al study's from 2019 if the pH is between 4.5 and 9. Additionally, Moeller (2017) revealed similar findings by pointing out that the urine pH should fall within the range of 4.5 and 8 in order to conduct an accurate drug test and prevent any adulteration. According to Casavant, (2002), if the pH of the urine sample is less than 3, it should be questioned. There have also been allegations of persons adulterating urine samples with substances like Tetrahydrozoline eye drops to generate a false-negative result for THC (Moeller et al., 2008). A urine specimen was announced as counterfeited if the pH was <3 or >11. A bogus specimen is any sample comprehending a matter that is not a normal ingredient of urine or containing an endogenous matter not present at an average non-pathological concentration. Adulterants may cause the specimen's pH to rise or fall, which might affect the immunoassay's binding and reaction rates. The targeted analytes may be less soluble in the urine matrix as a result of pH changes. The effectiveness and stability of analyte

extraction might be impacted by a change in specimen pH (Fu, 2019).

In terms of how adulterants affect urine creatinine levels, while the supplement of HCl bleach at a concentration of 10 or water dramatically reduced the creatinine level. There was a greater reduction in creatinine at concentration 40 when using 5% acetic acid, Tetrahydrozoline, HCl bleach, and water dilution; the highest significance was seen with double water dilution, followed by HCl bleach. Although HCl bleach's creatinine level considerably fell, it was still over the acceptable range of 20 mg/dl.

These findings are roughly in line with those of Moeller (2017), who said that the normal urine creatinine level should exceed 20 mg/dl. Additionally, Beck et al. (2000) reported same findings in their research. In their study, Cone et al. (1998) showed that 2 hours after consuming too much fluid, creatinine levels fell below the limit, which explains why the level fell following water dilution. Another research by Kim et al. (2019) examined the use of synthetic urine (SU) to evade drug testing. In their study, they claimed that SU are designed to have a pH between 5.5 and 7.5 and a creatinine concentration of at least 2 g/mL. This clarifies the possible cause of the on-site AdultaCheck® 6 strips failure to distinguish between the SU and real urine. According to Sara et al. (2016), urinary creatinine values under 20 mg/dl possibly indicate a tampered sample or the inclusion of an additional drug.

A dilute specimen is a urine specimen that contains creatinine but is lower than predicted for human pee, like in the Lin et al., (2018) research, where the creatinine content was recorded as dilute when it was 2 mg/dL but 20 mg/dL. When the original and confirmatory creatinine tests both showed a creatinine concentration of less than 2 mg/dL, a urine sample was reported as replaced. A urine sample containing creatinine that is sufficiently decreased or divergent that it deviates from typical human urine is referred to as a substituted specimen.

The urine creatinine is frequently reported by laboratories, allowing the doctor to decide the possibility of urinary dilution. Because the kidneys concentrate urine, drug metabolites are raised in it; as a result, urine concentricity of nominated medications and their metabolites are often

expressed as divided by urine creatinine. For instance, THC may be excreted by kidneys for up to thirty days after most contemporary use in heavy users (Grotenhermen, 2004). Urine specimens that test positive for THC must be neatly read to differentiate between continuous excretion and fresh usage. To resolve whether the creatinine-divided THC concentration is rising or falling with subsequent urine specimens. The urine THC concentration ought to be normalized by urine creatinine concentration. These proportions can then be matched with nomograms of THC excretion to fashion a clinical clearance (Schwilke et al., 2011).

Urinary creatinine according to Sara *et al.*, (2016) should also be recorded and creatinine levels minimal than 20 mg/dl may offer a modulated specimen or the supplement of another matter.

Regarding the impact of adulterants on the specific gravity of urine, the investigation revealed that HCL-based bleach at concentration 40 and twofold water dilution caused the largest, most significant drop in specific gravity (P-value 0.001).

In the study of Lin et al., (2018); a urine specimen was proclaimed as dilute when the specific gravity was >1.0010 but <1.0030 on a solitary aliquot. The specific gravity of a diluted urine specimen is lower than the anticipated for human urine. A urine specimen was considered as substituted when the estimated specific gravity was <1.0010 or >1.0200 on both the rudimentary and confirmatory specific gravity investigations using a refractometer on two disperse aliquots. A specific gravity of a substituted urine specimen is so lessen or swerved that they are not in the same line with normal human urine.

This is in line with Mikkelsen et al 's study (1988), which demonstrated that the average specific gravity of pure urine should be between 1005 and 1030. Additionally, the findings are consistent with those made public by Moeller (2017) and Beck et al. (2000), who showed that adulteration reduced urine's specific gravity to less than 1003.

In the current study; except for HCL-based bleach, which included nitrates in 100% of cases at a concentration of 40 and in 76.7% of cases at a concentration of 10, no nitrates were detected in urine samples before the addition of adulterants, according to this study.

Numerous ways, such as vicarious ones where the adulterant upsets the immunoassay precept, such as through pH or ion ferocity changes, or direct ways of the adulterant with the employed antibody or enzymes, might make the drug detection inaccurate. In addition, because of their changed chemical identities, oxidizing chemical adulterants may straightway oxidize and destroy goal analytes, making them impossible to detect by confirmatory assays (Steuer et al., 2017).

This could be explained by Unic *et al.* (2018) who studied changes that could occur during the use of urine strips. They revealed in their study that people treated by vitamin C may have large quantities of ascorbic acid (AA) in their urine. AA is known to collide with the thoroughness of some detecting test strips, causing bogus lower concentrations or negative results. Paradigms of investigations that may be confounded are the urine dipsticks for glucose, blood, bilirubin, nitrite, and glucose through the following reaction.

$$H2O2 + \text{Chromogen} \rightarrow \text{Oxidized chromogen (colored)} + H2O$$

The other part of the current study is comparing TLC, accurate card and VIVA E auto-analyzer immunoassay, that was done through validation of all of them and measuring SP, SN, PPV and NPV before and after adding adulterants comparing to GC/MS as gold standard for THC. Regarding the validation of the Accurate Card, VIVA E analyzer immunoassay and TLC before adding adulterants compared to GC/MS as a gold standard for THC detection, VIVA E immunoassay overall accuracy is better than other methods.

Dolan et al (2004) study revealed that cross reactivity may happen if an over-the-counter treatment or a prevalent environmental chemical partake chemical traits with the target analyte. This manipulates the results and can result in false positive results, which could account for the difference between specificity and PPV. In addition, Moeller et al. (2008) noted that the three urinalysis techniques include liquid chromatography (most expensive and most reliable), and radioimmunoassay, which is more expensive and sensitive, enzyme/ immunoassay, which is less expensive and less sensitive. There are two types of immunoassay methods: laboratory-based and point-of-collection (POC). Enzyme-multiplied immunoassay technique (EMIT), fluorescence

polarization immunoassay (FPIA), immunoturbidimetric test, and radioimmunoassay (RIA) are some of the several immunoassay methods (Sara *al.*, 2016).

In this study; the overall accuracy was the best for VIVA E immunoassay before adulteration, after adding 5% citric acid at 10% and 40% dilution, and Tetrahydrozoline eye drops at 10% and 40% dilution. The overall accuracy was the best for TLC after adding HCL bleach at 10% and 40% dilution and after dilution with water by 100% and 300%.; so overall accuracy is the best for VIVA E immunoassay except in HCL bleach at 40% and water dilution by 300%, TLC is better .

According to the current findings, several researches have indicated that HCL bleach can degrade analytes for GC/MS and interfere with immunoassay results. Tetrahydrozoline in Visine eye drops and acetic acid like vinegar both reduce the immunoassay's sensitivity to THC. Diuretics and water dilute the analyte to below the cutoff level (Ward et al., 2014; Weaver et al., 2015;Fu, 2016 ;Maas et al., 2017; Klega and Keehbauch, 2018).

Among the most often used adulterants are certain oxidizing compounds like nitrites, glutaraldehyde, chromates, and halogens like bleach and iodine; this result in a drop in sensitivity and NPV, as was shown at (Drugs.com, 2019). Currently, automated immunoassay is the method of choice for urine drug testing, either employed as a point-of-care test on its own or as the first step of a two-stage testing process. Qualitative immunoassay results are produced (i.e. a drug or its metabolite is evidence either present or absent, with no quantity appraisal). A screening immunoassay is followed by a confirming GC/MS in the two-step method (ASAM, 2013).

A second portion of the same sample is then put through a confirmatory GC-MS test for any chemicals that showed up as positive in the original immunoassay, with negative findings from the immunoassay (IA) being ignored. IA is frequently utilized as a point-of-care test due to its accessibility, affordability, and comparatively quick turnaround time. Most at-home urine drug test kits make use of immunoassay. Due to cross-reactivity, which occurs when chemicals in the biological material other than the real substrate or its metabolite bind to the test and provide a false-positive result, immunoassay

has lower specificity than GC-MS while having higher sensitivity. Additionally, standalone immunoassay drug tests cannot discriminate between medications of the same class (ASAM, 2013; Levy et al., 2014).

The components of thin layer chromatography (TLC) need little electrical power, are straightforward, inexpensive, and simple to use. For instance, the separation process can be completed with a hand-operated sprayer when indicator chemicals are utilized for visibility. However, electricity is needed when an ultraviolet (UV) lamp is employed. TLC is quick and can analyze many samples at once. With the right modifications, it may even be used in the field (Mwankuna et al.,2022)

V-CONCLUSION:

If urine drug testing is to be undertaken, pH, Specific gravity, urinary creatinine and nitrite presence should be valued and distrusted specimens should be rejected. Not all adulterants can be disclosed, so keep an eye on collection is strongly recommended. VIVA E immunoassay overall accuracy is better than other methods comparing to GC/MS as gold standard for THC before adding adulterants.

Exogenous chemical addition may result in a false-negative outcome, according to Fu (2019). In order to stop the surge in urine counterfeiting, toxicology laboratories have a number of defending measures and probity tests to detect the presence of sample manipulations. While these methods are quite effective in detecting adulterants, they typically fail to identify the specific pharmaceuticals that have been consumed. This explains why the methods for drug detection outlined above are losing accuracy. Unusual test results might indicate that the sample was intentionally tampered with or that they were a false negative.

VI-RECOMMENDATIONS:

It is recommended to evaluate pH, Specific gravity, urinary creatinine and nitrite presence in tests for illegal drugs. Urine samples that are suspect should be disregarded, and fresh samples should be taken. It is highly advised to collect urine samples under monitored conditions because urine samples can be successfully falsified and not all adulterants can be recognized.

VII-Declarations:

1. Ethics approval and consent to participate

Urine Samples were analyzed as secondary data analysis; so informed consent from cases is not applied. Ethical approval was obtained from the ethical committee of scientific research, Faculty of Medicine, Beni-Suef University, Approval number (FMBSUREC/05032023/Abdelaziz).

2. Consent for publication

Applicable

3. Availability of data and material

Applicable

4. Competing interests

None

5. Funding

The manuscript did not receive any fund for this research

6. Authors' contributions

All authors read and approved the final manuscript.

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تأثير العديد من مواد غش تحاليل المواد المخدرة في البول على العينات الإيجابية لوجود مادة الحشيش باستخدام الاختبارات المسحية

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الخلفية: يعتبر الغش ، وهو عملية تنطوي على التلاعب بعينة من البول بمواد كيميائية مغشوشة للحصول على نتيجة اختبار سلبية كاذبة ، أحد أكبر المشاكل في اختبار المواد المخدرة في البول. الهدف: تقييم تأثيرات بعض مواد غش تحاليل المواد المخدرة في البول على اختبار وجود مادة رباعي هيدروكانابينول (THC) في البول ، ومقارنة التأثيرات على طريقتين من طرق الفحص المناعي وكروماتوجرافيا الطبقة الرقيقة (TLC). الطريقة: تم اختبار عينات البول أولاً إيجابية لـ THC بواسطة GC / MS في معمل السموم بجامعة بني سويف. تم بعد ذلك غش عينات البول الموجبة لـ THC بواسطة الماء ، ومبيض HCL ، وحمض الخليك بنسبة 5 ٪ ، وقطرات Tetrahydrozoline للعين بتركيزات مختلفة. أخيرًا تم إعادة تقييم عينات البول من أجل THC باستخدام 2 من المقايسات المناعية ، وتم أيضًا تقييمه باستخدام TLC ، ودرجة الحموضة البولية ، والجاذبية النوعية ، والكرياتينين والنتريت. النتائج: انخفض الرقم الهيدروجيني للبول بشكل ملحوظ بعد غشائه بحمض أسيتيك بنسبة 5٪ بتركيزات 10 و 40 ، وزيادة ملحوظة في PH بعد 40٪ تتراهيدروزولين ، 10٪ و 40٪ حمض الهيدروكلوريك ، وبعد تخفيف الماء بنسبة 300٪. لم يكن لحمض الخليك بنسبة 5٪ ولا تتراهيدروزولين بتركيز 10 أي تأثير على مستويات الكرياتينين في البول. تسبب التبييض المستند إلى HCL بتركيز 40 وتخفيف الماء المزدوج في أكبر انخفاض في الجاذبية النوعية. باستثناء مادة التبييض المعتمدة على HCL ، والتي اشتملت على النترات في 100٪ من الحالات بتركيز 40 وفي 76.7٪ من الحالات بتركيز 10 ، لم يتم الكشف عن أي نترات في عينات البول قبل إضافة المواد الزانية. الدقة الكلية هي الأفضل للمقايسة المناعية لـ VIVA E فيما عدا مبيض HCL بنسبة 40٪ وتخفيف الماء بنسبة 300٪ ، يكون TLC أفضل. **أظهرت هذه النتائج** أن الدقة الكلية للمقايسة المناعية VIVA E أفضل من الطرق الأخرى مقارنة بـ GC / MS كمعيار ذهبي لـ THC قبل إضافة المواد الزانية. الاستنتاجات: تسبب مبيض حمض الهيدروكلوريك في التغيير الأكثر بروزًا في معاملات البول. تعد الدقة الكلية للمقايسة المناعية VIVA E أفضل من الطرق الأخرى مقارنةً بـ GC / MS.