A COMPARATIVE ANALYSIS OF THREE DIFFERENT IMMUNOASSAY TECHNIQUES FOR SCREENING OF DRUGS OF ABUSE IN URINE AND THEIR CONFIRMATION USING GC-MS& HPLC-MS

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Submit Date 2021-10-08
Revise Date 2022-09-20
Accept Date 2022-05-24

ABSTRACT

Background: Immunoassay is a laboratory technique that identifies and quantifies the antibody or the antigen in a sample by using the binding between an antigen and the homologous antibody. Using immunoassay techniques in forensic toxicological laboratories is very crucial since they are easy, sensitive, and yield preliminary results. **Objectives:** This study intends to cross-check the accuracy of three immunoassay techniques; Randox Evidence, Siemens V-Twin, and Abbott Architect c-4000 as preliminary screening techniques for detection of drugs of abuse in urine by confirming the results using chromatographic techniques. A total of 919 random human urine samples were collected from the General Department of Forensic Sciences and Criminology in Dubai Police and run equally in all the three instruments known to be widely applied in the field of toxicology and forensic science laboratories across the world. They were checked for their capability and efficiency in screening drugs of abuse. Once the screening was done, the positive samples were confirmed for the detected drugs by using the extraction technique. The extracted samples were then analyzed for confirmation using Gas Chromatography-Mass Spectrometry and HPLC MS instrument where these drugs and their metabolites were identified. The results were then compared with the libraries database in the system hence confirming the study and its aim.

Results: The results of the study confirmed that all three instruments were capable of screening drugs of abuse, but it also depends on the kits and the programs. It was seen that V-Twin and Architect c4000 showed almost similar results using EMIT but Randox which is using Biochip Array Technology was able to screen more varieties of drugs of abuse and their subclasses which were not detected in the screening with EMIT. **Conclusion:** The study concluded that Randox is the best screening accurate method for the detection of drugs of abuse. Each of the three instruments has its advantages and disadvantages as well as its maintenance technique, requisite time, and validity tests. Confirmatory tests run after extraction in GC-MS and HPLC-MS should also be taken into consideration. This study can aid in directing the course of forensic casework.

Keywords: Drugs of abuse, immunoassay techniques, confirmatory tests.

INTRODUCTION

A drug is any constituent besides food that when injected, inhaled, smoked, absorbed through a patch on the skin, consumed, or dissolved under the tongue, leads to temporary psychological changes in the body. **(Saferstein, 1990)** Classification of pharmaceutical drugs is often based on their mode of action, chemical structure, usage as well as the mechanism of action. In addition to therapeutic use, drugs can be consumed and abused for recreation purposes. The recreational use of the drug is simply the use of a given drug (controlled, legal, or illegal) with the primary purpose of altering the central nervous system to create positive feelings and emotions such as the use of hallucinogen LSD, a psychoactive drug. **(Alan et al, 2005), (Gary, 2000)**

Commonly abused drugs include opiates (codeine, morphine, and heroin), cannabinoids

(marijuana), methamphetamine and amphetamines, barbiturates, benzodiazepine, methadone, tramadol, fentanyl, gamma-hydroxybutyrate (GHB), ketamine, synthetic cannabinoids (Spice, K2, ABCHM, ABPIN), buprenorphine (BUP), pregabalin, bath salt drugs, tricyclic antidepressants (TCA), etc.

Immunoassay is a laboratory technique that identifies and quantifies the antibody or antigen in a sample by using the binding between an antigen and the homologous antibody. Immunoassays can be defined as analysis techniques that make use of immune reaction between the antibody (Ab) and antigen (Ag) for the determination of either of the reactants in a given solution thereby yielding a measurable result. **(Michael L. Smith, 2000)** Immunoassay is a significant toll in concentration measurements or the presence of micro and macromolecules of the substances to be tested in fluids such as serum and urine using antibodies and antigens. The tests are majorly employed in postmortem investigations, sports anti-doping lab, and clinical pharmacokinetics and therapeutic monitoring of drugs among other areas. **(S. Christophersen et al, 2000)** Some types of immunoassays are lateral flow immunoassays, fluorescent polarized immunoassay (FPIA), fluorescent resonance energy transfer (FRET), radioimmunoassay (RIA), and enzyme-linked immunosorbent assay (ELISA). **(Marin et al, 2011)**

Adulteration is a process of purposefully tampering with a specimen to change the results of testing. Adulterants may cause "false negative" results by either interfering with the test or damaging the drug metabolites in the sample. **(Ricardo, 2014)**

To rule out adulteration, certain urine parameters that have to be measured such as:

• Creatinine: The presence of low creatinine value in urine can be due to two reasons. Either the person drank too much water before being tested to dilute the urine sample or water was added intentionally to the sample.

• Specific Gravity (Dilution): Creatinine levels are often used in combination with specific gravity to see if samples had been diluted. To avoid this problem, the on-site collector may color the toilet water blue to prevent sample dilution.

• PH (Acidity): Certain enzymes may be added to the urine sample to affect its stability, but this often changes the pH, which is also screened during the preliminary process of drug testing. Normal pH levels range from 4.0 to 9.0.

• Oxidants: This test is performed for detecting the presence of oxidizing agents such as bleach or hydrogen peroxide in the urine sample. Pyridinium Chlorochromate is a commonly used adulterant. Normal human urine samples do not contain any oxidants.

• Nitrates: A test that is commonly used for commercial adulterants such as Klear or Whizzes. Since any natural urine sample should not have any traces of nitrite, positive results for nitrate indicate an adulterant presence. **(Tsai, S-CJ, et al, 1998)**

• Glutaraldehyde: Adulterants such as Uric acid and Clear Choice contain glutaraldehyde which may cause false-negative screening results by interfering with the enzyme used in different immunoassay tests. Glutaraldehyde is not normally found in urine; so its detection in a sample indicates tampering. **(Ricardo, 2014)**.

Cheating testing of drugs could be achieved through various methods which include:

1. Substituting the urine sample with synthetic urine or drug-free urine purchased from a clandestine source that is not easily detectable because synthetic urine has the same chemical parameters as normal urine. Cortisol is a specific test that could be used to detect compounds that are normally found in human urine but absent in synthetic urine.

2. Drinking a commercially available product to flush out drugs that usually contain caffeine or other diuretics to increase the output of urine. The aim is to produce a diluted urine sample so the concentration of drugs of abuse and or their metabolites will drop down the recommended cutoff level. (Flushing-Detoxification agents- Diuretics)**.**

3. In vitro adding of an adulterant to the urine specimen after collection: ex. Cannabinoid and opiate assay are susceptible to bleach.

4. PCP and benzoylecgonine analysis are affected by alkaline agents. When interpreting drug results, the positivity of oxidants should be taken into consideration as they may decrease the level of some drugs or produce false-negative results either by masking the drug's presence or by damaging the drug sample. **(Ricardo, 2014)**

Problems and challenges during examination:

False-negative results may be due to sample adulteration and very high drug test cut-off level.

False positive results: **(Kirschbaum, Katrin M., et al, 2011)**

Causes:

1. Cross-reactivity of compounds similar to the drug constituents.
2. Carryover from the previous sample.
3. Using very low cut-off levels in some laboratories.
4. Second-hand smoke from marijuana. **(David T, 2000)**

False-positive tests are always of concern as indignant donors normally want to prove their innocence. However, the false-negative results are more important as they pose greater safety risks because despite having consumed illicit substances, no one who gets a false-negative drug test will complain about the result. **(Barry Levine, 2010)**

There are two types of techniques that can be used for the detection of drugs of abuse which are presumptive and confirmatory tests, each has its advantages and disadvantages.

The presumptive techniques include:

1: EMIT assay (V-TWIN) uses an enzyme-linked antigen. The substrate glucose-6-phosphate (G6P) is oxidized to glucuronolactone-6-phosphate by the enzyme glucose-6-phosphate dehydrogenase (G6P-DH). Additionally, G6PDH reduces nicotine amide adenine dinucleotide to NADH. This is monitored spectrophotometrically by measuring the absorbance of NADH produced at a wavelength of 340 nm.

The enzymatic activity of G6PDH decreases due to the attachment of the drug to the antibody. Therefore, adding the drug causes the labeled drug to be released from the antibody which in turn increases the production rate of NADH. Hence, the concentration of the drug is directly proportional to the change in absorbance at 340 nm. **(Allen et al, 2005)** This application is used for preliminary detection of ten groups of drugs of abuse in urine samples (opiates, benzodiazepines, barbiturates, cocaine, propoxyphene, methadone, PCP, amphetamine, THC, and tramadol) including urine validation (adulteration) (creatinine, pH, and specific gravity). **(Allen et al, 2005)**

2-ARCHITECT C4000: It uses ready-to-use liquid reagents. To detect the drug in the urine, monoclonal antibodies are used in the assay. The assay is based on competition between the drug from the urine and an enzyme-labeled drug for a fixed number of specific antibody binding sites. The specific antibody binds to the drug labeled with G6PDH in the absence of the drug from the sample. This causes the enzyme activity to be inhibited. The enzymatic activity of glucose-6-phosphate is determined spectroscopically at 340/412 nm due to its ability to reduce NAD to NADH. This application, ARCHITECTC4000, is used for preliminary detection of ten groups of drugs of abuse in urine samples (Opiates, Benzodiazepines, Barbiturates, Cocaine, Propoxyphene, Methadone, PCP (Phencyclidine), Amphetamine, THC, Tramadol and Spice) including urine validation (adulteration) in which it tests for creatinine, pH and specific gravity. A more specific chemical method must be used however since this assay only provides preliminary analytical results only. Examples of preferred confirmatory methods include mass spectrometry and gas chromatography.

3-Randox evidence: The Randox Evidence analyzer is a fully automated, continuous access immune analyzer using protein Biochip array technology. It is used in the semi-qualitative, fully qualitative, and in-vitro determination of various diagnostic markers. Immunoassays are done on the surface of a Biochip. This is then taken to several treatment stations within the analyzer. The final process results in the production of light from a chemiluminescent reaction. The light generated is measured using a charge-coupled device (CCD) camera. The light output from discrete test regions on the Biochip surface is quantified using imaging technology. Randox Evidence is used for preliminary detection of ten groups of drugs of abuse in urine samples. It also tests for creatinine in the process of urine validation. A confirmatory method is preferred to get a confirmed analytical test. Examples of this include mass spectrometry and gas chromatography. This is because random evidence alone provides preliminary analytical results only.

The confirmatory tests include:

1-Gas chromatography-mass spectrometry (GC–MS) instrument is applied to detect compounds using the relative gas chromatographic retention times and elution patterns of components of a mixture in combination with the mass spectral fragmentation patterns, and the compound's chemical structures. **(Alan et al, 2005)**

2-HPLC is a type of chromatography whose function is to pump an analyte mixture into a

solvent at high pressure via pillar chromatography with a filling solid or a motionless stage. **(AK Jaiswal. Tabin Millo, 2014)**

Objectives of the study:

This study aimed to compare the accuracy of three immunological assays (Randox Evidence, EMIT system (v-twin), and Architect c4000 (Abbott) and to conclude the most accurate one in drug detection in urine.

Aims:

- Screening of humane urine samples for drugs using (Randox Evidence, EMIT system (v-twin), and Architect c4000 (Abbott).
- Determine the most accurate preliminary techniques for drug detection in urine.
- Confirm results of screening techniques by using HPLC-MS and GC-MS.

MATERIALS & METHODS:

Sample Collection: (**AK Jaiswal and Tabin Millo, 2014)**

The research was conducted in the toxicological lab of Dubai Police in UAE after taking the ethical approval from the responsible committee and signing the informed consent forms. Although there are various samples that can be collected from the human body to be used in drug testing, like hair, sweat, saliva, blood, urine, etc.… the preferred biological sample is urine due to being easy to collect, available in more quantity, having a higher concentration of drug than any other sample from the same specimen and being easy for detection of adulteration

A total of 919 urine samples were collected from law offenders and drug addicts with an age range between 15 – 50 years. The samples were labeled in series and stored at low temperatures before being run in the instruments for comparison. The samples were set aside for comparison and the same batch was run one time each for screening of drug of abuse in the instruments. Several tests are available to validate samples like PH, Specific Gravity, and Creatinine, which are run along with the drug of abuse screening.

Before the samples were tested, a control check was done for all the DOA and validation tests to see if all the readings were falling in the required range confirming the instrument to be in perfect working condition. Various other factors should be taken into consideration before screening the sample for DOA like knowing the cutoff value of the drug you are looking for, running controls, and calibrations to ensure if the instrument is working perfectly so as one can trust the results of the immunoassay.

Despite all the controls and calibrations results being perfect and the instrument running smoothly, know that there can be false-positive and false-negative results. The reason for such is that the traces from previous highly concentrated samples can contaminate the needle causing a carryover. Also due to the presence of other substances with the same structure in the sample, a false positive can be expected.

The reason for false negatives could be the manipulation of the sample by adding acid, base, or water to change the dilution and concentration levels of the sample.

The known ranges normally for a standard urine sample should be:

- PH - between (4-10)
- Specific gravity - more than 1.003
- Creatinine - approximately 20mg/dL or more

Note that if the levels of creatinine are between the ranges (5-20 mg/dL), then the sample is possibly diluted and if the range is below 5mg/dL then the sample may not be urine at all. The 919 urine samples were collected, and tests were performed as follows:

Extracted urine samples that tested positive were analyzed on (Randox Evidence, EMIT system (v-twin), and Architect c4000 (Abbott). The principle of V-Twin and Architect c4000 instruments EMIT assays is an enzyme-linked antigen and Randox Evidence uses Biochip Array Technology.

The enzymatic activity of G6PDH is decreased when the attached drug is bound to the antibody, so the addition of drugs causes the labeled drug to be released from the antibody and increases the rate of NADH production. The change in absorbance at 400 nm in while 340 nm is directly proportional to the concentration of the drug in the sample.

Specific safe work practices were followed for the individual instruments according to the manufacturing safety guidelines of the instrument in use. In addition, periodical maintenance was performed by testing the safety of the individual instrument. An Agilent GC-MS with DB-5MS Ultra inert column (30 m x 0.25 mm x 0.25 µm) and split-less mode were used. The initial temperature of the oven was maintained at 70 °C and then increased to 280 °C at a rate of 12°C/min, where it was held constant

for 11 min. The total flow, purge flow, and pressure were set at 24 mL/min, 3 ml/min, and 8.8085 psi, respectively. The mass spectrometer used for detection had a scan range set from 50-550, ion source temperature set at 230°C ,and quadrupole temperature set at 150°C. There are positive samples that appeared on the Randox Evidence only and did not appear on the other preliminary examination Instruments (EMIT system (v-twin) & Architect c4000 (Abbott) because they did not contain the kits designated for examining these narcotics for example fentanyl. The results of these narcotics were confirmed using HPLC-MS.

Extracted urine samples that tested positive were analyzed on (Randox Evidence only, using LXQ ion trap mass spectrometer instrument.

Table (1): LC.MS conditions

Instrument	LXQ ion trap mass spectrometer
Ionization	ESI, Thermo Scientific Ion Max source
Capillary temperature	275℃
Spray voltage	5.0 kV
Sheath gas	30
Aux gas	8
Data acquisition mode	Polarity switching scan dependent experiment
Microscans	1
Wide Band Activation	On
Stepped Normalized Collision Energy	35% ± 10%

Sample Preparation:

-The turbid sample was centrifuged at 4000 rpm for 1 minute before the examination.

-Small number of samples (1-2 ml) were poured into labeled instrument tubes.

Preparation of apparatus: Before running the control and the calibration for the instrument daily maintenance was carried out.

Sample Extraction:

Extraction of samples was done using both Liquid-liquid extraction, and Solid-phase extraction.

All positive samples were confirmed using different extraction methods.

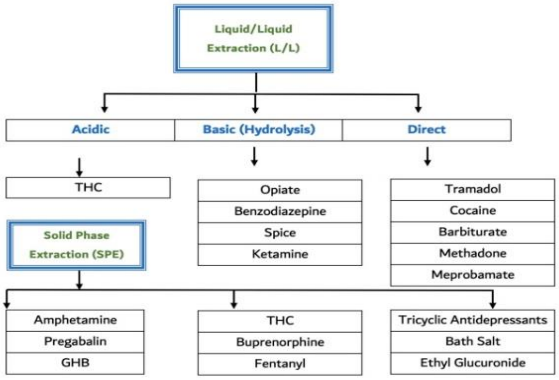

Figure (1): Types of extraction

Confirmatory Analysis:

All positive samples were confirmed using GC-MS and HPLC- MS. The confirmation of the screening tests was done via extraction and further running the analysis in Gas Chromatography and Mass Spectrometry as well as some of the analysis was done using HPLC-MS. Once the preliminary tests were performed, the extraction method for the detected drug of abuse was applied. The extractions used were liquid-liquid extraction, which was further split into basic and acidic extraction. For a few others like Amphetamine and Pregabalin, a solid-phase extraction technique was used along with using the columns to separate the extract and run into the specific instruments. Once these extracts were run in the instruments, the GC-MS column helped in further separation as per retention time and atomic masses of the compound. Showing clear peaks which were later compared with the database libraries and further confirming the presence of the respective drug and its metabolite as shown in the screening results.

GC-MS instrumentation and usage: (Marin, Stephanie J., et al, 2009)

Once the extraction was completed, the extract of the sample was run into Gas Mass Chromatography and HPLC-MS for analysis and interpretation of the results. For example: The method used to run the Pregabalin is direct. Once the run was complete, the results could be found in the library in W10N14.L. Note that for all internal standards added, the library search run is TOXI-LAB.L. The major ions to find the Pregabalin peak are 102, 288, 210, and 225, which can be inserted for a quick search. If the extraction is done well, and the internal standard Cod-D3 (ion 374 - TMS) is found; the other peak for Pregabalin will appear in the search, hence confirming the presence of the drug of abuse in the sample.

Table (2): Pregabalin result findings and interpretation

Method	Direct method	
Library	W10N14.L	TOXI-LAB.L
Major Ions	**102, 288**, 210,225	374
Findings	Pregabalin TMS	Cod-D3 TMS

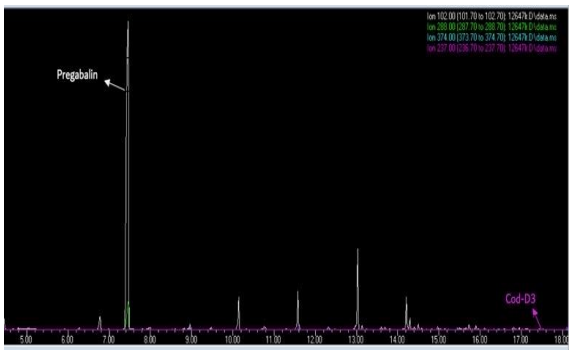

Figure (2): Pregabalin result findings and interpretation

The method used to run the tramadol is the direct method and also the opiate method as an alternative. Once the run was complete, we could find the results in the library in AAFSDRUG.L library for direct method and PMW_Tox3.I for Opiate method. Note that for all internal standards added, the library search run is TOXI-LAB.L. The major ions to find the Tramadol and its metabolites peak are 263, 279, and 249, which can be inserted for quick search. If the extraction is done well, and the internal standard Diazepam D5 (ion 287) is found; the other peaks for tramadol (OH) (ion 279) will appear in the TOXI-LAB.L search, Desmethyl Tramadol (N) and Desmethyl Tramadol (O) (ion: 249) will show in PMW_Tox3.I search Tramadol (ion: 263) using the direct method in AAFSDRUG.L search; hence confirming the presence of the Drug of abuse in the sample.

Table 3: Tramadol result findings and interpretation

Method	Direct method	Opiate method		
Library	AAFSDRUG.L	PMW_Tox3.I	TOXI-LAB.L	
Major Ions	263	249	279	287
Findings	Tramadol	Desmethyl Tramadol (N) Desmethyl Tramadol (O)	Tramadol (OH)	Diazepam D5

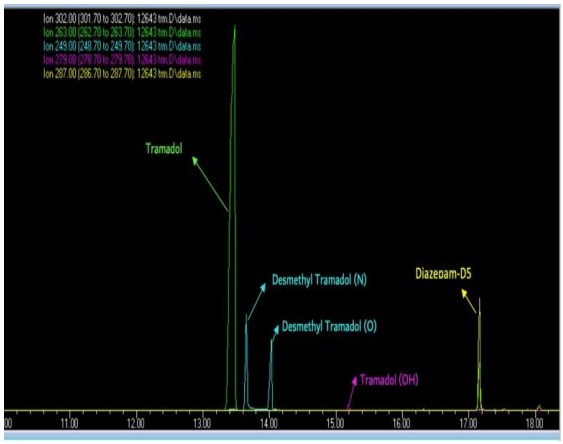

Figure (3): Tramadol result findings and interpretation

RESULTS:

In this research, a total of nine drugs were screened in 919 urine samples using three different screening methods. The tests were done on three separate instruments which are known to be widely applied in the field of toxicology and forensic science laboratories across the world. These instruments are known to offer screening using enzyme multiple immunoassay technique (EMIT) and Biochip Array Technology – linked immunosorbent assay. The performance, results, and efficiency of the three instruments used in the detection and screening of drugs of abuse in this study were:

Table (4): Keywords of drug names

CODE	DRUG NAME		CODE	DRUG NAME
COC	Cocaine	**3**	FENT	Fentanyl
THC	Cannabinoids	**4**	MPB	Meprobamate
AMP	Amphetamine	**5**	KET	Ketamine
MAMP	Methamphetamine	**6**	BUP	Buprenorphine
PGB	Pregabalin	**7**	ETG	Ethyl Glucuronide
MAM	6-Monoacetylmorphine	**8**	TCA	Tricyclic Antidepressants
OP	Opiates	**9**	ABPIN	ABPIN (spice)
BEZ	Benzodiazepines	**0**	ABCHM	ABCHM (spice)
TRAM	Tramadol	**1**	UR-144	UR-144 (spice)
MDON	Methadone	**2**	CR	Creatinine
BATH	Bath Salt	**3**	SG	Specific Gravity
BARB	Barbiturates	**4**	PH	PH

1 – V-Twin:

This instrument is widely used in screening drugs of abuse in most forensic laboratories due to being user-friendly and efficient. When the 919 samples were screened through Twin Viva, it was noticed that out of 919 samples a total of 108 were found positive for Cannabinoids (9-carboxy-11-nor-Δ^9-THC), and around 33 positives for tramadol as shown in table 5 and figure 4, which is widely abused. 121 urine samples were screened positive for Pregabalin, a drug that is marketed under the brand name Lyrica and used in epilepsy, generalized anxiety, neuropathic pain, etc. Cocaine is also known as coke, showed 23 positive results during screening. Methadone which is widely used in opioid maintenance therapy in opioid dependence patients was seen to be positive in 6 urine samples as shown in table 5 and figure 4. Whereas opiate or opioid was seen to be positive in 41 samples as compared to benzodiazepines which are a class of psychoactive drugs, showed positive results for 22 urine samples. The most widely positive or popular during the screening was Amphetamines, which are Central Nervous System (CNS) stimulants used to treat a variety of conditions but also highly taken for recreational use, with a total count being 137 out of 919 samples. But there were no results found positive for barbiturates. The count for barbiturates was 0 out of 919 urine sample screening. Along with these drug of abuse tests, validity tests were run for creatinine and specific gravity each with 95 as shown in table 5 and figure 4. It was recorded that out of 919 samples run for screening, 585 came out as negative with no drug of abuse detected as shown in n table 6 and figure 8.

Table (5): V-TWIN preliminary results

V-twin preliminary results for (919) samples													Total Samples
THC	TRAM	PGB	AMP	BARB	COC	MDON	OP	BEZ	CR	SG	PH	NEGATIVE	919
108	33	121	137	0	23	6	41	22	95	95	0	585	

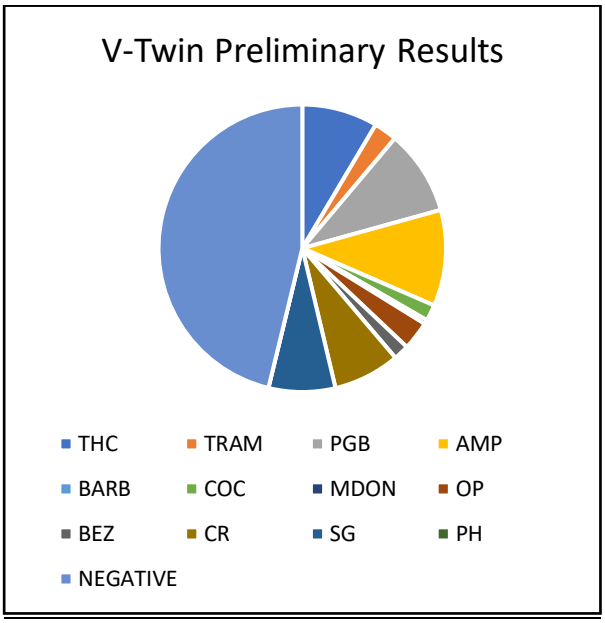

Figure (4): V-TWIN preliminary results

Table (6): Negative preliminary results

Negative preliminary results for (919) samples			
V-TWIN (CR–PH – SG)	ARCHITECTc 4000(CR–PH – SG)	RANDOX (CR)	Total Results
585	559	439	

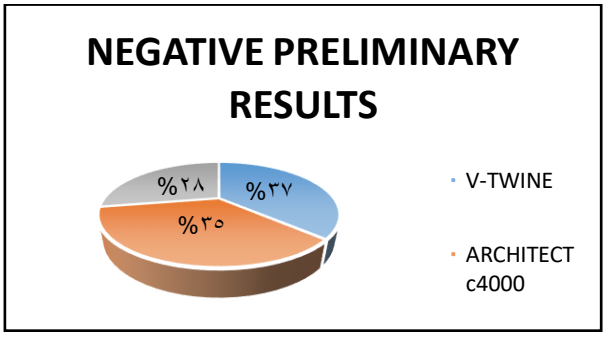

Figure (5): Negative preliminary results

Table (7): V-Twin confirmatory results

V-TWIN results confirmation by using Gas Chromatography Mass Spectrometry for (919) samples											Total Samples
THC	TRAM	PGB	AMP	BARB	COC	MDON	OP	BEZ	False positive	NEGATIVE	
105	33	121	132	0	23	3	39	18	17	585	919

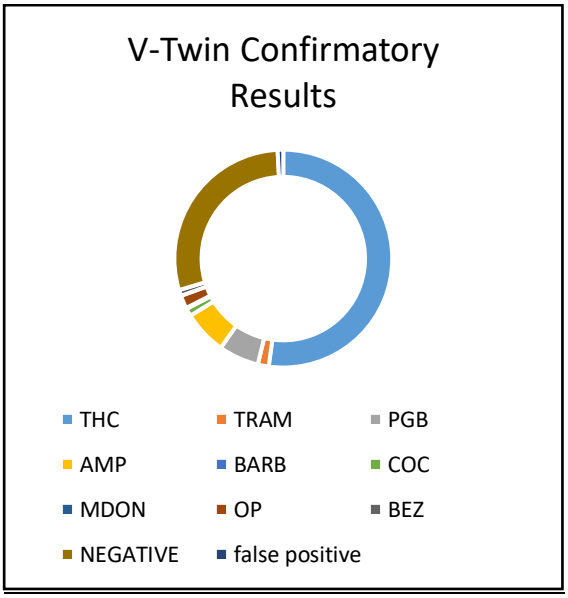

Figure (6): V-Twin confirmatory results

2 – Architect c4000:

The very same set of these 919 selected urine samples was run for another screening, using a different instrument but the same principle. This instrument is by Abbott and is called Architect c4000. It uses Enzyme Multiple Immunoassay Technique also known as EMIT. It is known for its rapid demonstration and high accuracy result testing. In its many featured offerings, it's well-known also for its rapid STAT turnaround time. The results of screening drug abuse in 919 samples were observed. It was seen that out of all the samples, a total of 118 urine samples showed positive results for Cannabinoids (9-carboxy-11-nor-Δ^9-THC) as shown in table 8 and figure 7. It is a chemical found in cannabis. Further other drugs were detected, like Tramadol being screened positive for 31 samples, similarly a higher value for Pregabalin, giving around 133 urine samples as positive out of 919. But as earlier seen in V-Twin results, even here the high number of samples were detected positive for Amphetamine screening, giving a large margin result of 151. But unlike V-twin, one barbiturate positive sample was observed among 919 same urine samples for screening as shown in table 8 and figure 7. Cocaine was detected in about 9 samples, along with five positive screening tests for Methadone. Opiate showed positive for 34 samples and Benzodiazepines had a result of 22 positive urine samples during screening as shown in table 8 and figure 7. The same validity tests for Creatinine and specific gravity were done along with screening in this assay giving results for 107 samples each. Regarding negative samples, it was seen that out of 919 samples, 559 were displayed as negative for drug of abuse screening as shown in table 6 and figure 5.

Table (8): ARCHITECTc4000 preliminary results

Architectc4000 preliminary results for (919) samples											Total Samples		
THC	TRAM	PGB	AMP	BARB	COC	MDON	OP	BEZ	CR	SG	PH	NEGATIVE	919
118	31	133	151	1	19	5	34	22	107	107	0	559	

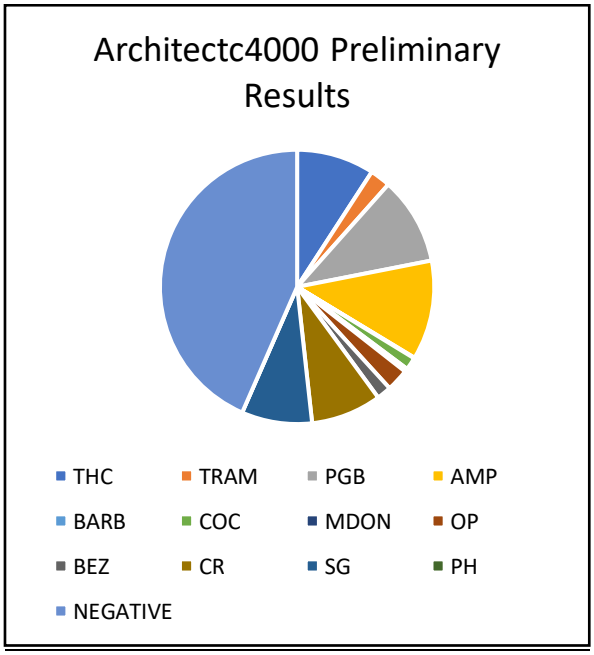

Figure (7): ARCHITECTc4000 preliminary results

Table (9): ARCHITECTc4000 confirmatory results

ARCHITECTc4000 results confirmation by using Gas Chromatography Mass Spectrometry for (919) samples										
THC	TRAM	PGB	AMP	BARB	COC	MDON	OP	BEZ	False positive	NEGATIVE
117	31	133	144	1	19	3	33	18	15	559

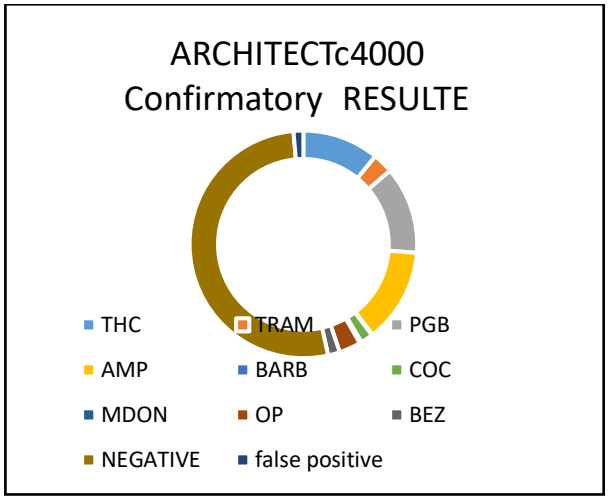

Figure (8): ARCHITECTc4000 confirmatory results

3 – Randox Evidence:

This instrument is used for rapid multiplex drug testing and its software is quite user-friendly like all the other instruments used for screening in this study. Further, the same 919 urine samples were run on this instrument using a multiplex testing platform allowing for the simultaneous quantitative or qualitative detection of a wide range of analytes from a single sample. The results were observed to be different than the first two instruments as it combines the Biochip Array Technology. It was surprisingly noticed that 4 samples were positive for Barbiturates as presented in table 7 and figure 6, as compared to none or one in the previous instruments. 3 samples were positive for Methadone and about 33 showed positive results for Tramadol. For benzodiazepines, about 29 samples were screened positive and for opiates, the number escalated to 39 as shown in table 10 and figure 9. In the case of pregabalin, the results went much higher, showing positive for 135 urine samples and for Cannabinoids (9-carboxy-11-nor-Δ^9-THC) 138 was the confirmatory figure as positive.

It was also seen that not just for Amphetamine like the other two instruments but also for methamphetamine the results were shown positive. These two drugs listed positive separately as 26 and 39 respectively for the screening of urine tests as shown in table 10 and figure 9.

It was observed that along with these predictable drugs, that were expected and found, there were surprise elements, and some new other drugs of abuse were highlighted in the results. For example, Bath Salts showed positive for 6 samples as presented in table 11 and figure 10, which was not seen in any other two instruments earlier. Fentanyl was recorded positive for 2 urine samples and showed no result in the other two instruments. Likewise, a spice which is also known as synthetic cannabinoid was screened positive for 3 urine samples in Randox Evidence as shown in table 11 and figure 10.

Other than these, more unexpected drugs like Tricyclic antidepressants were also found positive for 43 urine samples. Ethyl glucuronide which is a metabolite of ethanol found in the body after glucuronidation due to the consumption of alcohol beverage was also recorded positive in 95 urine samples as presented in table 11 and figure 10. Another such drug, buprenorphine, which is mostly sold under the brand name Subutex, falling in the opioid use disorder or chronic pain was shown as positive in 21 urine samples. But Ketamine which is widely used in starting and maintaining anesthesia, causing trance state, sedation, and pain relief was found in 4 samples.

21 samples stood positive for Meprobamate which is used as an anxiolytic drug. With so many positive samples for different drugs of

abuse, the negative samples were considered, and 439 samples were recorded as negative out of 919 urine samples run for the comparison screening test.

Further on comparison of the results between these 3 instruments based on their capacity, performance, and efficiency of screening, it was noticed that in the case of Cannabinoids (9-carboxy-11-nor-Δ^9-THC), the V-Twin results showed 108 out of 919 positives urine samples for the drug Cannabis as shown in table 5 and figure 4 whereas the Architect c4000 from Abbot showed 118 positive results for the same as shown in table 8 and figure 7, which further compared to Randox Evidence gave a much higher as presented in table 10 and figure 9. 138 Cannabinoids (9-carboxy-11-nor-Δ^9-THC) positive results which were later confirmed by using extraction method and analyzing the results with discovery of THC metabolite.

But with the cases of Tramadol urine screening, it was noticed that V-Twin from Siemens and Evidence from Randox out of 919 samples showed 33 positives for the Tramadol which was slightly lower than Architect which showed a result of 31 positive for tramadol.

Pregabalin results were more due to the sensitive nature of the drug of abuse as well as the instrument that the sample was run on. Out of 919 urine samples, V-twin showed a result of 121 positives for Pregabalin which when compared to Architect c4000 gave a much higher value as 133 positive results for Pregabalin, which in comparison to Randox gave a slightly higher figure of 135 positive results for the same drug. These analytical procedures such as sampling, the addition of reagents, and measuring of both were all carried out in an automated system.

Amphetamine results were seen most high for Architect c4000, which is out of 919 a total of 151 samples showed positive for the drug of the abuse whereas in Randox and V-twin the positive figures were almost the same 135 and 137 respectively showing a minor difference. With this, it was observed that architect is the most appropriate instrument for Amphetamine screening as it gave more positive results, but Randox was seen to give separately 26 and 39 as positive results for amphetamine and methamphetamine respectively. These results were confirmed by GCMS.

The comparison of Barbiturates in the three different instruments was surprising, as V-twin showed no results for barbiturates whereas Architect c4000 gave one positive result for the drug out of 919 samples as presented in table 8 and figure 7 and Randox gave out 4 positives for the Barbiturates as shown in table 10 and figure 9 showing its more sensitivity compared to the other two. The urine specimens that screened positive by any method were confirmed and quantitated by gas chromatography/mass spectrometry (GC/MS) as presented in tables 7, 9, and 13.

Cocaine results were not very variably different but rather with a low margin for all the three instruments. This was done using capabilities of different analytical techniques which involves, sampling, extraction, and purification of cocaine and its metabolite. Giving a result out of 919 samples run, 23 were positive for cocaine in V-Twin, 19 positives for cocaine in Architect c4000, and 26 cocaine positive results in Randox. These results from three different instruments were confirmed by GCMS. Further stating architect as the least sensitive for cocaine screening. The metabolite of cocaine which is benzoylecgonine was identified during analysis after extraction.

Methadone and opiate results were as follows, out of 919 samples, 6 samples were found positive for methadone in V-Twin as shown in 5, 5 in Architect as presented in tables 8 and 3 in Randox as shown in 10. Whereas for Opiates the figures were 41, 34, 53 for V-Twin, Architect c4000, and Randox respectively. The 53 positives for opiates showed that the biochip assay was more sensitive when compared with the other two for opioids.

Benzodiazepines which are widely abused antidepressants showed a positive result of 22 samples in V-Twin as well as Architect c4000 but different in Randox with the positive Benzodiazpines being higher as 29 out of 919 samples. This shows the low sensitivity of benzodiazepines in V-Twin.

In the case of validity tests, all three instruments performed well as per their efficiency. It was seen that in V-twin and Architect c4000 as presented in tables 5 and 8 the validity tests consisted of Creatinine, pH, and specific gravity whereas in randox only available validity test was Creatinine as seen in table 10. So performance was rated accordingly. It was observed that all the validity tests run for the three instruments did not have much variation in comparison to their results. But all three instruments must have all the three validity tests

along with the screening to detect any kind of adulteration and sample tampering. But it should be common to accept small rates of false-positive in forensic cases.

That being said, there were a few drugs that showed reading only in Randox and not the other two instruments which was a great advantage for Randox as its working efficiency was much better due to the various kits available and designed differently for more drug abuse screening as is shown in table 12 and figure 10. These urine samples were confirmed using LC/MS, and their chromatograms were inspected for any presence of drug or related product as shown in table 14 and figure 11.

For example, the other two instruments were not equipped for the tests that Randox showed positive like Spice (synthetic cannabis), Tricyclic antidepressant (cyclic antidepressants), Buprenorphine (Subutex), Ethyl Glucuronide (a metabolite of ethanol), Ketamine (anesthetic), Fentanyl, Bath Salt and Meprobamate (anxiolytic). This shows that the biochip assay in Randox detected more specimens that were not detected by ELISA and the results were confirmed using GCMS as presented in table 13. Results for spice were confirmed using LC/MS as shown in table 14 by comparison of results peaks revealing quantitative differences.

Finally, when comparing the negative samples as shown in table 6 and figure 5, out of 919, V-Twin and Architect c4000 showed 585 and 559 Negative samples respectively whereas Randox Evidence rounded the figure up with 439 Negative samples which were the lowest negative samples as compared to the other two hence proving that the Randox Evidence screening has more advantage over the other two instruments in screening and detection of most drug of abuse by being more efficient and more sensitive. With great accuracy and low negative results.

It was also observed that out of eleven samples, five were found positive for GHB during extraction. These urine samples were confirmed negative using the immunoassay technique as the instruments used for screening have specific kits and software programming for only certain drug of abuse detection. A small rate of false positives can be accepted in forensic cases. A special request was made in one of the cases where they found a dropper bottle containing colorless unknown solution on the person. Further analysis of the solution confirmed it to be containing GHB&GLP by the chemistry dept. The samples were requested for GHB extraction by the authority. Since the result of screening was negative, these samples were directly moved through the extraction procedure and it was confirmed that 11 of them were positive for GHB in GC-MS.

Table (10): RANDOX EVIDENCE preliminary results (1)

RANDOX EVIDENCE preliminary results for (919) samples (1)												
BARB	BATH	MDON	TRAM	BEZ	OP	MAM	PGB	MAMP	AMP	THC	BZG (COC)	Total Results
4	6	3	33	29	39	0	135	39	26	138	26	
					14			70				
					53			135				

Table (11): RANDOX EVIDENCE preliminary results (2)

RANDOX EVIDENCE preliminary for (919) samples (2)											
FENT	CR	UR-144	ABCHIM	ABPIN	TCA	ETG	BUP	KET	MPB	NEGATIVE	Total Results
2	89	0	1	2	43	95	21	4	21	439	

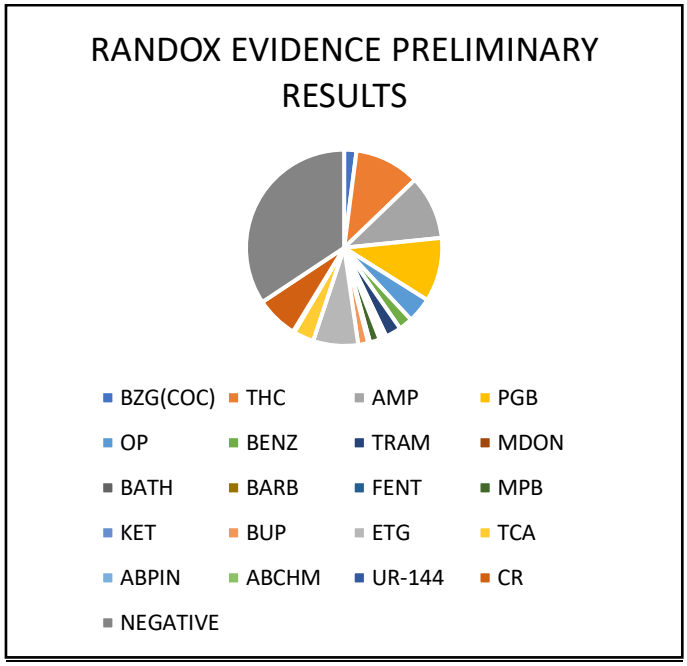

Figure (9): RANDOX EVIDENCE preliminary results

Table (12): Drug results only available on RANDOX EVIDENCE

Drug preliminary results are only available on RANDOX EVIDENCE for (919) samples										
UR-144	ABCHIM	ABPIN	TCA	ETG	BUP	KET	MPB	FENT	BATH	Total Result
	1	2	43	95	21	4	21	2	6	

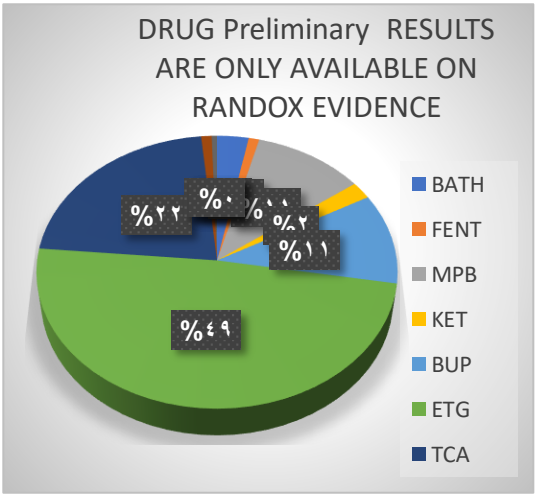

Figure (10): Drug results on RANDOX EVIDENCE

Table (13): RANDOX results confirmation by using Gas Chromatography Mass Spectrometry

RANDOX RESULTES confirmation by using liquid chromatography mass spectrometry for (919) samples											
FENT	BATH	UR-144	ABCHIM	ABPIN	TCA	ETG	BUP	KET	False positive	NEGATIVE	
2	6	0	1	0	40	90	20	4	11	439	Total Results

Table (14): RANDOX results confirmation by using Liquid Chromatography Mass Spectrometry

RANDOX results confirmation by using Gas chromatography Mass Spectrometry for (919) samples													
BARB	MPB	MDON	TRAM	BEZ	OP	MAM	PGB	MAMP	AMP	THC	BZG (COC)	False positive	
4	18	3	33	26	37	0	135	37	25	137	26	14	Total Results
					14			68					
					51			130					

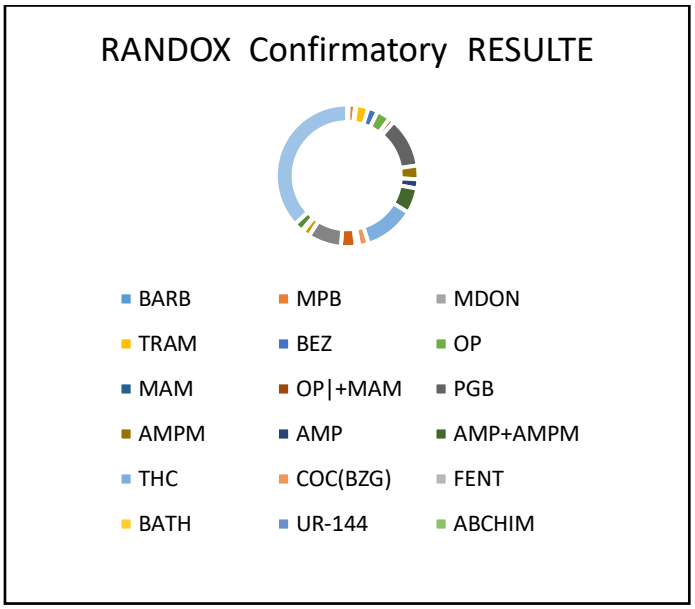

Figure (11): RANDOX confirmatory results

DISCUSSION

This study showed how at times certain drugs (that aren't programmed into the three preliminary screening test instruments) can go undetected when the sample is positive for the drug but due to the limited kits and programming it cannot be seen hence showing the sample as negative. Such cases were confirmed as positive with a confirmatory test. It was seen that V-Twin and Architect c4000 both showed almost similar results during the tests using EMIT but Randox which is using ELISA gave better results screening more drugs of abuse which was not seen in the screening with EMIT. Being said that evidence from Randox uses the Biochip Array Technology which uniquely offers immunoassay diagnostic testing for simultaneous multi-analyte biomarker detection. Each biochip has up to 49 Discrete Test Regions (DTR's) each detecting a different biomarker, making the instrument highly sensitive and preferable in drug of abuse screening. It is the world's first Biochip Array Technology system (BAT) and has a sample capacity of 180 and up to 44 analytes screened per biochip. The drawback though is in Randox samples run in bulk or batch would be of greater advantage than the singular load or a fewer sample. For example, once the instrument starts a run, the loading module gets locked and no more samples are allowed to be loaded until the previous run gets done and results are given out. In addition, the results for Randox are not given out as they are processed but instead collectively by the end of completing all the samples which can cause a delay in result collection. Another drawback of Randox is, the Instrument takes a longer period for daily maintenance and control checks. This can cause a delay in urgent cases of screening. Hence although Randox is the best option for screening drugs of abuse, it should be noted that it works great at hospitals or places where samples are run in batches and do not come first screen first basis.

Hence when comparing the two other instruments, the V-Twin from Siemens, and Abbot Architect c4000 using the same Enzyme Multiple Immunoassay technique as well as same working and principle along with similar kits and working, it was seen that out of 919 sample tests run in both these instruments, we

have achieved better results in screening and more positive samples for Drug of Abuse in Architect c4000. However, the drawback of the V-Twin is its daily maintenance, which requires constant refilling manually of the reagents and kits, which can delay the process of screening. The calibration and controls run check at times can cause an error and the air bubbles during aspiration can give out-of-range results, not to mention the carryover risks showing false positive and false negative which can be avoided in Randox. The only disadvantage in Abbot Architect c4000 was the racks that are used to insert sample cups are all barcoded and a slight error in the series or printing and manufacturing of these racks or their barcodes can cause the failure of detection of the racks by the instrument scanner causing the tests not to run and giving an error which can lead to delay in screening.

CONCLUSION

It was concluded that all the three instruments were capable and efficient in screening drugs of abuse efficiently as per their principle and working, but it also majorly depends on the kits and programming provided. Keeping all the comparison in mind, the advantages and disadvantages of the three instruments and the screening results in terms of drug of abuse, maintenance, and time is taken, validity tests, etc... as well as the confirmatory tests, run after extraction in GC-MS and HPLC-MS, it is concluded that all the three instruments have their good points as well as drawbacks, but Randox Evidence yielded most accurate results in terms of screening drugs of abuse in the total of 919 samples; giving and screening more variety of subclasses and substances that were not screened using the EMIT.

RECOMMENDATIONS

1-The preliminary and confirmatory tests could be tried with other categories of drugs of abuse as they are very broad.

2-Different new preliminary and confirmatory tests and techniques could be tried, checked, and compared for their accuracy in detecting drugs of abuse, especially the immunoassay techniques.

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دراسة مقارنة لثلاث تقنيات مناعية مختلفة لفحص المخدرات في عينات البول وتأكيد النتائج باستخدام كروماتوغرافيا الغاز المزود بمطياف الكتلة (GC-MS) والاستشراب السائل المزود بمقياس طيف الكتلة (HPLC-MS).

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الملخص:
المقايسة المناعية هي تقنية معملية تحدد الجسم المضاد أو المستضد في عينة باستخدام الارتباط بين مولد الضد والجسم المضاد المتماثل. يعد استخدام تقنيات المقايسة المناعية في مختبرات الطب الشرعي للسموم أمرًا بالغ الأهمية لأنها سهلة وحساسة وتعطي نتائج أولية. هناك فئة واسعة من تقنيات المقايسة المناعية والأجهزة المستخدمة في تحقيقات الطب الشرعي. تهدف هذه الدراسة إلى التحقق من دقة ثلاث تقنيات للمقايسة المناعية Randox Evidence و Siemens V-Twin و Abbott Architect c-4000 ، كطرق فحص أولية للكشف عن تعاطي المخدرات في البول من خلال تأكيد النتائج باستخدام تقنيات الكروماتوغرافيا. تم جمع 919 عينة عشوائية من البول البشري من الإدارة العامة لعلوم الطب الشرعي وعلم الجريمة في شرطة دبي وتشغيلها بالتساوي في جميع الأدوات الثلاثة المعروفة بتطبيقها على نطاق واسع في مجال علم السموم ومختبرات الطب الشرعي في جميع أنحاء العالم. تم فحصهم لقدرتهم وكفاءتهم في فحص تعاطي المخدرات. بمجرد الانتهاء من الفحص ، تم تأكيد العينات الإيجابية للأدوية المكتشفة باستخدام تقنية الاستخلاص. تم إجراء طرق الاستخلاص هذه بناءً على الطبيعة الحمضية والأساسية للدواء بالإضافة إلى تقنية الاستخلاص في المرحلة السائلة أو الصلبة ، ثم تم تحليل العينات المستخرجة للتأكيد باستخدام مقياس الطيف الكتلي للكتلة الغازية وجهاز HPLC MS حيث تكون هذه الأدوية تم تحديد نواتجها باستخدام الكتل الذرية والأيونات في أوقات الاحتفاظ المحددة. ثم تمت مقارنة النتائج مع قاعدة بيانات المكتبات في النظام ومن ثم تأكيد الدراسة وهدفها من مقارنة الأدوات الثلاثة بناءً على تحليل نتائج للكشف الأولي عن تسع مجموعات من المخدرات. خلصت الدراسة إلى أن جميع الأدوات الثلاثة كانت قادرة وفعالة في فحص تعاطي المخدرات ولكنها تعتمد أيضًا بشكل كبير على الأدوات والبرامج المقدمة. جميع الأدوات الثلاثة لها مزاياها وعيوبها. وقد لوحظ أن كلا من V-Twin و Architect c4000 أظهروا نتائج دقيقة متشابهة تقريبًا أثناء الاختبارات باستخدام EMIT ولكن Randox التي تستخدم Biochip Array Technology تمكنت من فحص المزيد من أنواع العقاقير المخدرة والفئات الفرعية التي لم يتم اكتشافها في الفحص باستخدام EMIT ، ومن هنا استنتج أنها أفضل طريقة فحص دقيقة ويرجع ذلك إلى تقنية Biochip Array Technology، التي تقدم بشكل فريد اختبارًا تشخيصيًا للمقايسة المناعية لاكتشاف العلامات الحيوية المتعددة التحليلات المتزامنة. تم أخذ مزايا وعيوب الأدوات الثلاثة ، نتائج الفحص من حيث تعاطي المخدرات ، الصيانة ، الوقت المستغرق ، اختبارات الصلاحية وكذلك الاختبارات التأكيدية التي أجريت بعد الاستخراج في GC-MS , HPLC-MS في الاعتبار . يمكن أن تساعد هذه الدراسة في توجيه مسار أعمال الطب الشرعي.

الكلمات المفتاحية: تعاطي المخدرات ، تقنيات المقايسة المناعية ، الاختبارات التأكيديّة