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Review on The Effects of Adulterants on Drugabuse Testing in Urine Samples

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Abstract

Introduction: A growing concern over the use of illicit drugs in the work place has led to an interest in urine analysis as a way to detect drug abuse. Sample adulteration is a serious potential problem in forensic urine drug testing. Federal guidelines define an adulterated specimen as a urine specimen containing a substance that is not a normal constituent or containing an endogenous substance at a concentration that is not a normal physiologic concentration. Adulterants act by either interfering with immunoassay procedures or by converting the target drugs to other compounds. Once the adulterants are converted to other compounds they do not bind to the antibodies used in immunoassay. In some cases these converted compound produce false negative results in confirmatory testing. Adulterants can be classified into two categories. The first category includes in vivo adulteration comprising intentional ingestion of fluids, substances or drugs designed to dilute urine. The second category includes in vitro adulteration such as common household chemicals and nitrite containing agents. Methods of detection of urine adulterants include urine integrity tests, color tests and spectrophotometric methods.

Key words Drug of abuse, urine analysis, adulteration.

Introduction

In the last years, a growing concern over the use of illicit drugs in the work place has led to an interest in urine analysis as a way to detect and deter drug use.

Drug testing by urine analysis has been suggested and in many cases implemented for prospective and current employees in industry; for personnel of the armed forces; for parolees and bail seekers in civilian court systems; for workers in the transportation industry; and for individuals who serve as role models, such as nationally known athletes (Hadland & Levy 2016).

Two factors have led to the widespread use of urine analysis for drugs: technical developments in testing methods and the growing demand for drug testing. Society is becoming increasingly aware of the impact of drug abuse on public safety and the financial impact on industry of lost time and productivity. The annual loss of productivity of employees has been estimated at 100 billion$ for alcohol and drug abuse, a third of which is due to drug abuse alone (Fu, 2016).

Sample adulteration is a serious potential problem in forensic urine drug testing. An adulteration can be defined as any process by which an individual knowingly interferes with (or attempts to interfere with) the processes of specimen collection, transport or analysis with the intention of avoiding a legitimate test result (Olivieri et al., 2018).

A variety of substances have been employed to interfere with testing procedures in hopes of causing the sample to yield a negative result (Dasgupta, 2007).

Federal guidelines define an adulterated specimen as a urine specimen containing a substance that is not a normal constituent or containing an endogenous substance at a concentration that is not a normal physiologic concentration (Bush, 2008).

I. Mechanism of action for adulterants:

- **Photometric interferences**: Causing an optical interference during analysis.
- **Alterations of pH**: Most enzymes have a narrow pH range by which activity is optimized (vinegar, lemon juice, bicarbonate, and alkaline detergents)
- **Antibody-antigen interactions**: All immunoassays rely on specific interaction between antibodies from the assay and the antigens (drugs) from the specimens. This delicate balance can be disrupted by changes in pH, ionic strength, viscosity, and surface tension.
- **Miscellaneous interactions**: Benzalkonium chloride (which present in Visine eye drops) promotes the sequestration of tetrahydrocanabinol (THC) into micelle bodies, making it unavailable for binding to THC specific antibodies (Dasgupta, 2010).

II. Forms of adulteration:

II.1.Substitution:

Substitution includes the practice by which a urine specimen from a drug abusing donor is switched by urine from a drug free individual (Moeller, 2008).

Two procedures are currently in use for detection of substituted urine.

A- Monitoring urine temperature immediately after the collection - Unfortunately, acceptable temperatures can be achieved if the substituted urine is stored in the axilla, vaginal cavity, or next to the scrotum just prior to donation (Lee et al., 2013).

B- Careful witnessing of the collection of urine itself. Unfortunately, effective same gender witnessed collection, requiring close observation of urination, which is an unpleasant duty for most individuals. Even with close observation where the individual is closely monitored during a void, substitution can still occur. The donor can conceal a pouch of drug free urine and release its contents directly to the urine cup (Lee et al., 2013).

II.2.In vivo adulteration:

II.2.1.Dilutional method: The intentional ingestion of fluids, substances, and/or drugs designed to dilute urine or to hasten or increase the metabolism and/or excretion of drugs in the body (Mladěnka et al., 2018).

*Water is an effective in vivo dilution adulterant. In case of psychogenic polydipsia the patient routinely consumes large volumes of water, which can dilute urine or electrolytes by up to ten fold. Drugs will present at or near the cut-off and negative results can be produced (Fraser & Zamecnik, 2003).

Phencyclidine (PCP) and THC are not excreted in very high concentrations into urine so can be diluted by excessive fluid intake (Luzzi et al., 2004).

Other drugs such as opiates and cocaine produce drug concentrations that can exceed ten times the cutoff concentrations, particularly when urine is donated soon after the drug is used. For these individuals, dilutional adulterants may not be effective in producing the desired negative result because it is not possible to consume enough water to reduce urine drug concentrations to that extent (Drummer, 2006).

**Diuretics are drugs used for the treatment of heart failure, hypertension, hepatic ascites, pulmonary edema, and renal edema. There are many types of natural and synthetic diuretics. It should be noted that some diuretics give the urine an unusual color, indicating the presence of an unnatural condition (Qavi et al., 2015).

***Xanthine compounds are diuretics found in popular beverages like caffeine (coffee and tea), theophilline (tea) and theobromine (cocoa). Xanthines increase blood flow to the kidneys and may produce a diuretic action through an increase in the glomerular filtration pressure (Singh et al., 2018).

****Food or liquids that are highly acidic (such as vinegar) or basic (such as sodium bicarbonate) can produce sufficient changes in urine pH to interfere with immunoassay screening procedures. Acidification or alkalization can affect the metabolism and rate of clearance for drugs (Mirrakhimov et al., 2017). For example, Amphetamine as a basic drug, 74%of the parent compound is excreted in urine that is slightly acidic. In alkaline urine (intake of sodium bicarbonate/baking soda), only 1% is excreted as the parent compound. In a similar way, acidification has been reported to enhance the excretion of weakly basic drugs (Lin & Strathmann, 2013).

II.2.2. Miscellaneous drugs and substances:

There are many other drugs and substances that can cause negative interferences with drugs that could be considered candidates for an in vivo adulterant by a drug abuser. Compounds like salicylates (aspirin), ibuprofen, and fluorescein (used in retinal angiography) can invalidate the test such that a re-collection may be required. Thus the drug-positive individual may escape detection on that particular instance and might abstain from use before recollection (Soycan, 2019).

II.3.In vitro adulterants:

II.3.1 Common household chemicals as urinary adulterants.

Household items and over the counter pharmaceutical products are popular in vitro adulterants since they are readily available in bathroom closets, pockets, and purses (Oliveri et al., 2018)

Several adulterants can cause false-negative results in drug testing by immunoassays. Common adulterants for masking drug testing are as table salt, household vinegar, liquid laundry bleach, concentrated lemon juice, goldenseal tea, dettol [chloroxylenol], Pearl hand soap, ethanol, isopropanol and eye drops (Dasgupta, 2007).

II.3.2.Adulteration of urine with nitrite containing agents:

Potassium nitrite (Klear) dissolves in urine without affecting color or temperature. Klear may cause a false-negative GC-MS confirmation result for marijuana (Jaffee et al., 2007).

Duration of nitrite exposure and the urine matrix also affect the THC-COOH assay. In an in vitro study, 40 clinical urine specimens confirmed as positive for THC-COOH were supplemented with 1.15 or 0.30 mol/L of nitrite. The results indicated that the pH of the urine and the original drug concentrations have major roles indicate the effectiveness of nitrite in causing false negative THC metabolite test results (Tsai et al., 2000).

Whizzies is another urine adulterant available from the Internet. This adulterant also contains potassium nitrite (Dasgupta et al., 2004).

II.3.3.Stealth as an urinary adulterant:

Stealth is an adulterant advertised as an effective way to escape detection in a urine drug test. Stealth consists of two vials, one containing a powder (peroxidase) and another containing a liquid (hydrogen peroxide). Both products are added to the urine specimen. Stealth is capable of producing false-negative results using immunoassay methods when marijuana metabolites, lysergic acid diethylamide, and opiates (morphine) are

present in the urine at 125% to 150% of cutoff values (Fu, 2016).

Adulteration of an authentic positive sample provided by a marijuana user caused the sample to screen as negative with these immunoassay reagents. A low concentration of morphine (2,500 ng/mL) could be effectively masked by Stealth, but a higher concentration (6,000ng/mL) tested positive by immunoassay. GC-MS confirmation can be affected if Stealth is present in the urine**.** Unfortunately, a routine specimen integrity tests did not detect the presence of Stealth in urine (Charlton et al., 2014).

II.3.4.-Glutaraldehyde as an adulterant of urine:

Glutaraldehyde has also been used as an adulterant to alter urine drug test results. This product is available under the trade name (Urine Aid). Each kit contains 4 to 5 mL of glutaraldehyde solution, which is added to 50 to 60 ml of urine (Dasgupta, 2010).

Glutaraldehyde solutions are available in hospitals and clinics as a cleaning or sterilizing agent. A 10% solution of glutaraldehyde is available from pharmacies as over-the-counter medication for treatment of warts (Lipke, 2006).

Glutaraldehyde at a concentration of 0.75% by volume can lead to false-negative screening results for a cannabinoid test using the Enzyme Multiplied Immunoassay Technique (EMIT) II drugs-of-abuse screen (Syva). Amphetamine, methadone, benzodiazepine, opiate, and cocaine metabolite tests can be affected at glutaraldehyde concentrations between 1% and 2% with EMIT immunoassays. At a concentration of 2% by volume, the assay of cocaine metabolites is significantly affected (Dasgupta, 2010).

II.3.5.Urine Luck as a urinary adulterant:

The active ingredient of Urine Luck is 200 mmol/l of pyridinium chlorochromate (PCC). It was reported that a decrease in the response rate for all EMIT II drug screens and for the Abuscreen morphine and THC assays. In contrast, Abuscreen amphetamine assays produced a higher response, and no effect was observed on the results of benzoylecgonine and PCP. This adulteration of urine did not alter GC-MS confirmation test results for methamphetamine, benzoylecgonine, and PCP. However, apparent concentrations of opiates and THC as determined by GC-MS were reduced (Charlton et al., 2014).

Methods of detection of urine adulterants

1-Urine integrity tests

Specimen integrity tests involve monitoring urine parameters such as PH (4-10), specific gravity (1005-1025), and creatinine levels (20-400 mg/dl). These simple parameters form part of endogenous urine characteristics. Any significant deviation from the expected values observed from specimen integrity tests may indicate urine manipulation (Fu 2016).

2- Color tests

Spot tests give rapid results and are simple to perform. PCC, nitrite, and Stealth can be detected using various color spot tests. Cr6+ in PCC can be detected by taking 1 mL of urine and adding two drops of a 1% 1,5-diphenylcarbazide solution in methanol (w/v) and observing any color change. A reddish purple color indicates a positive result (Dasgupta, 2010).

Nitrite can be detected by using acidified potassium permanganate, a reagent that is pink in color. Samples containing nitrite will be immediately discolored and effervescence is observed when the reagent is added. Stealth in urine detected by an immediate color change to dark brown upon addition of a solution of tetra-methylbenzidine with 100 mM phosphate buffer, this indicative of a positive result. Another reagent, acidified potassium dichromate, will give a deep blue color change that fades over time when exposed to urine containing stealth (Caitlin et al., 2007). (Wu, 2003) described a simple fluorometric method for the detection of glutaraldehyde in urine. When 0.5 mL of urine was heated with 1.0 ml of a 7.7-mmol/L concentration of potassium dihydrogen phosphate (pH3.0) saturated with di-ethylthiobarbituric acid for one hour at 96°C to 98°C in a heating block, a yellow-green fluorophore developed if glutaraldehyde was present.

3-Dipstick devices

Dipstick devices are portable and allow for on-site testing to detect adulterated specimens. Due to this advantage, there are many devices commercially available. Typically, these involve plastic strips with chemically treated pads affixed, with each pad being an assay for different specimen attributes. The MASK Ultrascreen (Kacey Inc.) device contains a large range of assays in its testing panel and can test for creatinine, pH, specific gravity, nitrite, glutaraldehyde, PCC, and Stealth, but only at high concentrations, which are well above the concentrations found in urine following the recommended usage of the adulterants (Kuzhiumparambil and Fu, 2013).

Designed for forensic toxicological purposes, the Adultacheck 4 and 6 (Sciteck® Diagnostics) can be used to test urine specimens for creatinine, PH, glutaraldehyde and PCC, over large ranges and so are able to detect unusually high or low levels. Similarly, the Intect®7 (Branan Medical Corporation), reportedly the most sensitive and economical device available, can detect creatinine and pH over a wide range (Dasgupta et al., 2004).

4-Spectrophotometric Methods

Spectrophotometric analysis is an accepted analytical tool for studying peroxidase enzyme activity and may be used to analyze urine specimens for the presence of Stealth. Six additional spectrophotometric methods have been developed for the purposes of detecting oxidants in urine which include ferric, chromate, nitrite, permanganate, oxychloride, and hydrogen peroxide. Although a number of these methods exist, they are not currently part of routine testing protocols, presumably due to cost (Paul, 2004).

5-Immunoassay

An immunoassay-based test specifically used for the detection of urine adulteration by oxidizing compounds is the Microgenics DRI® performed on an automated

clinical chemistry analyzer; this assay is based on the reaction between a tetra-methylbenzidine reagent and any oxidant present in the specimen. The reaction results in a colored complex able to be observed at 660 nm. Similar to the spectrophotometric methods, this assay appears to not be implemented in routine drug testing laboratories mainly due to cost (Fu, 2016).

6-Capillary Electrophoresis and Electrospray Ionization–MS

Capillary electrophoresis has been used to detect the chromate ions found in PCC and nitrite ions found in nitrite-based oxidants. The chromium species present in PCC can also be detected using mass spectrometric techniques such as LC–MS, GC–MS, and inductively coupled plasma–MS (ICP–MS). HPLC coupled to MS or a conductivity detector has been demonstrated to detect the active Cr6+ and nitrite ions in commercial urine adulterants (Minakata et al., 2008).

7-Polyethylene glycol urine marker system.

The marker system involves the ingestion of biologically inert low-molecular-weight polyethylene glycols that are excreted in the urine after oral ingestion. This allows reliable specimen identification by the successful detection of the specific marker substance in urine samples. It is important to note that the method is effective in detecting urine substitution based on the absence of the polyethylene glycol marker in the urine specimens. However, the method is not effective in detecting in vitro adulteration by chemicals, as chemical adulterants can be added into the urine specimens after voiding (Schneider et al., 2008).

Role of chromatography in drug abuse testing

It would be ideal to bypass immunoassay screens and perform a more sensitive and specific confirmatory test on all specimens to avoid the limitations of immunoassays in particular the proplem of adulteration (Melanson et al., 2012).

There are a number of established chromatographic techniques available to clinical and forensic toxicologists, such as thin-layer chromatography (TLC), gas chromatography (GC), and high-performance liquid chromatography (HPLC) (Coskun, 2016).

1. Chromatographic techniques have many advantage in drugabuse testing as:
2. High resolution, good repeatability
3. High sensitivity (ppm-ppb) and accurate quantitative measurements.
4. Less thermal and catalytic decomposition of sensitive sample components.
5. Detect the pyridinium chlorochromate and nitrite ions in commercial urine adulterants (Yogesh et al., 2018).

Conclusion

To prevent false negative results, it is important to apply the appropriate urine collection procedure. Not to forget the importance of surveillance while obtaining the sample because it is hard to detect the adulterants in analytical and post-analytical phase. Specimen integrity tests can reduce false negative results. Also, in case of clinical suspicion, a validation test should be performed with confirmatory methods as chromatography.

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مقالة عن تأثير المواد الشائبة على تحاليل الكشف عن المخدرات فى عينات البول

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الملخص العربى

أدى القلق المتزايد بشأن تعاطى المخدرات إلى الاهتمام بتحليل البول كوسيلة للكشف عن المخدرات. يعتبر غش العينة مشكلة محتملة خطيرة في اختبارتعاطى المخدرات عن طريق البول، وتعرف عملية الغش طباقاً للمعايير الفدرالية بأنها التلاعب في عينة البول بقصد تغيير نتائج الاختباروبتم ذلك إما بالتدخل في إجراءات المقايسة المناعية أو عن طريق تحويل المركبات المستهدفة إلى مركبات أخرى.حيث انها بمجرد تحولها إلى مركبات أخرى ، فإنها لا ترتبط بالأجسام المضادة المستخدمة في المقايسة المناعية ، وفي بعض الحالات تنتج هذه المركبات المحولة نتائج سلبية خاطئة في الاختبار التأكيدي، وتنقسم المواد الشائبة إلى نوعين إما داخليا إما عن طريق شرب كمية من السوائل بغرض التخفيف أو استخدام مواد أو أدوية مصممة لهذا الغرض، و النوع الاخر يتم إضافته لعينة البول فى الخارج مثل المواد الكيميائية المنزلية الشائعة اوالمواد المحتوية على النيتريت.وتتضمن طرق الكشف عن المواد الشائبة اجراءاختبارات سلامة البول واختبارات الألوان وطرق القياس الطيفي.

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