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Research Article

How to Screen for Adulterated and Synthetic Urine Samples when Testing for Drugs of Abuse? A Study to Evaluate and Integrate Axiom Assay in Specimen Validity Testing Protocol

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Abstract

Objectives: Sample adulteration and synthetic urines are challenges laboratories need to address when testing for Drugs of Abuse. Creatinine, pH and specific gravity (SG) assays are commonly tested for specimen validity, but these assays are not sufficient to address sample adulteration and synthetic urines. In this study, we evaluated the Axiom assay as a part of specimen validity testing (SVT).

Methods: We analysed 2000 urine samples and five different synthetic urines to evaluate the Axiom assay. pH and SG were tested because they are integral to the result interpretation of the Axiom assay. Samples were also tested for creatinine and oxidants.

Results: Two Thousand patients' samples and five different synthetic urines were analysed. The Axiom assay result should be < 2 to be considered positive if SG is ≥ 1.004 and pH is ≤ 8.1. Creatinine was also analysed. The percentage of samples flagged as synthetic urine in our tested population is 4.4% (44 cases/1000 patients).

Conclusion: The Axiom assay detects four endogenous substances in urine and the outcome is interpreted alongside pH and SG results. The Axiom assay is recommended for testing with creatinine, pH, SG and oxidant assay, for screening both adulterated and synthetic urines. When urine samples are tested for sample integrity, if a sample is flagged because of this screening process, then this sample should be further tested by Axiom assay to detect synthetic urines. Also, the Axiom assay could be employed as one of the parameters tested in SVT.

Keywords: *Drugs of Abuse (DOA); DOA Screening; Specimen Validity; Synthetic Urine*

Abbreviations

Carboxy-THC: Carboxy-Tetrahydrocannabinol; GC-MS: Gas Chromatography/Mass Spectrometry; LC-MS/MS: Liquid Chromatography with Tandem Mass Spectrometry; N: Number of Specimens; SG: Specific Gravity; SVT: Specimen Validity Testing

Introduction

Urine drug screening is important for illicit drug testing, following up compliance for medication, abusing and diversion of prescribed drugs and medications, and testing employees to ensure safety in the workplace [1]. Some of the methods used for adulteration include dilution with water, substitution with a drug-free liquid, adding household liquids and/or chemicals such as detergents, vinegar and baking soda or using synthetic urine such as Urine-Aid, which contains glutaraldehyde Klear™, which contains potassium nitrite). Also, trying to change the urine pH to facilitate faster drug elimination such as phencyclidine, and amphetamines. Detecting a drug is dependent on the drug dose, metabolism, the drug half-life time and the drug cut-off level. Sometimes the sample is adulterated with chemicals such as bleach or substituted with synthetic urine or drug-free urine [2].

In drug testing, a laboratory should focus on two areas; the detection of an illicit substance and the possibility of a false-negative result due to sample adulteration or substitution. Drug compliance can be masked by different methods of adulteration which may include dilution with water, the addition of liquids such as vinegar, baking soda, liquid drain opener, and detergents. Another method of cheating a drug test is to substitute the urine with drug-free or use synthetic urine instead. A dilute urine specimen may give a false-negative result and consequently misinterpret results. There is a lot of knowledge on the internet regarding how a person can cheat to pass a drug test. The Substance Abuse and Mental Health Services Administration (SAMHSA) in the United States of America ruled that creatinine, SG, and pH were to be performed for specimen validity [3]. But such protocols do not address the issue of synthetic urine and its impact on presenting results and consequently misleading reports.

Each laboratory screening for drugs of abuse should establish a protocol in place for specimen validity testing (SVT) to address current challenges in this area. Our study evaluated the Axiom assay as part of the screening protocol.

A complete urine drug of abuse testing program normally involves specimen collection and initial screening with an immunoassay, followed by a confirmation test, such as LC-MS/MS (Liquid Chromatography with tandem mass spectrometry or Gas Chromatography/Mass Spectrometry (GC-MS), for the positive samples [4]. Many oxidizing adulterants are sold under the claim that they will clear positive drug test results. Examples include Nitrite such as Klear™, Chromate such as Urine Luck™, iodine, horseradish, bleach and Peroxidase (H_2O_2) such as Stealth™ [5]. When a urine sample is adulterated, there may not be a significant change to the sample appearance, pH, SG or creatinine concentration.

Materials and Methods

The reagents obtained from Thermo Fisher and their part numbers are pH-Detect Kit (CDF100054), pH Calibrator Set (CDF100283), pH 7 Control (CDF100284), pH 10 Control (CDF100285), Gravity Detect Kit (CDF1194), Gravity-Detect Low Calibrator (CDF1754), Gravity-Detect High Calibrator (CDF1755), Gravity-Detect Level 1 Control (CDF1756), Gravity-Detect Level 2 Control (CDF1757), creatinine-detect (CDF1797) and creatinine calibrator set (CDF100272). Creatinine controls are obtained from Bio-Rad, Liquicheck urine chemistry control levels 1 (397) and 2 (398). The creatinine method is based on the Jaffe reaction, whereby creatinine concentration is determined colourimetrically using alkaline picrate to form a reddish Janovski complex [6].

Axiom assay from Axiom Diagnostics was supplied by PM Separation (www.pmsep.com.au). Five different synthetic urines are also obtained from PM Separation. The Synthetic urines used in this study are P-sure, golden flask, clean stream, Dr John's Pee, and Dr Green's agent.

Axiom assay from axiom diagnostics is designed to detect synthetic urine by detecting four endogenous substances in the urine, which are not mentioned on the package insert of the assay to protect the assay from attempts to invalidate it. It is an endpoint colourimetric

assay measured at 340 and 600 nm wavelengths. A study conducted by the manufacturer of 4235 urines using Axiom assay, SG and pH assays yielded the following results. Two hundred thirty-seven (5.6%) had synthetic urine values < 2. One hundred eighty-nine of these samples had a specific gravity < 1.004, a pH value > 8.1 or both, and were classified as normal human urine. Of the remaining forty-eight samples, 34 produced significant precipitates and are therefore human urines. The remaining 14 urines (0.33%) are synthetic products. This assertion is substantiated by the lack of uric acid in 11 of the 14 samples.

In our study, we analysed 2000 urine samples and 5 different synthetic urines to evaluate the Axiom assay. pH and SG are tested too because they are integral to the result interpretation of the Axiom assay. Samples were tested for creatinine and oxidants using oxidant assay. AU5810 analyser was used for the above assays.

Results

Creatinine level was analysed for all samples. Two Thousand patients' samples were analysed plus five different synthetic urines. The axiom assay result should be < 2 to be considered positive if SG is ≥ 1.004 and pH is ≤ 8.1. The interpretation of the results is explained in table 1. These are the parameters identified by the manufacturer to be used to interpret the outcome of the axiom assay.

Axiom assay result	SG result	pH result	Conclusion
< 2	≥ 1.004	≤ 8.1	Synthetic Urine
≥ 2			Normal Human Urine
< 2	< 1.004		Normal Human Urine
< 2		> 8.1	Normal Human Urine

Table 1: *Interpretation of axiom assay result in conjunction of SG and pH according to the kit manufacturer.*

The breakdown of the total number and ratio of patient samples flagged as synthetic urine is 4.4% (44 patients in every 1000 patients), as shown in table 2.

Source of 2000 Samples Analysed	Number of Flagged Samples	Ratio of Flagged Samples
Drug Court Samples	26 out of 2000 samples	(26/2000) x 100 = 1.30%
Clinic Samples	62 out of 2000 samples	(62/2000) x 100 = 3.10%
Total	88 out of 2000 samples	(88/2000) x 100 = 4.4%

Table 2: *Number and ratio of samples flagged as synthetic urine based on the analysis of 2000 samples.*

The projection of the possible number of samples flagged as synthetic urine per year based on the analysis of 2000 samples is summarised in table 3.

Clinics	Number of Samples Flagged and Percentage of the 2000 Tested Samples	Number of samples Tested/year	Predicated number of samples to be flagged as synthetic urine/year
Clinic 1	26 Samples (1.3%)	26,485	344
Clinic 2	62 Samples (3.1%)	27,263	845
Total	4.4%	53,748	1,189

Table 3: *Projection of possible number of samples flagged as synthetic urine per year based on the analysis of 2000 samples.*

Creatinine, pH and SG cut-off values and relevant interpretation are summarised in table 3. Although urea was not analysed, the target values for urea are also mentioned in table 4 for reference.

Creatinine Decision point* (mmol/L)	Creatinine Concentration	Specific Gravity g/mL	pH	Urea mmol/L	Report Comment
≥ 1.768 mmol/L	Normal	1.003 - 1.035	4.5 to 9.0		Normal urine.
			> 9 and < 9.5		May indicate poor storage condition and/or Urinary Tract Infection (UTI).
≥ 0.44 and < 1.76	Dilute	1.0011 - 1.0029	> 9.0 but < 11.0	< 50	Dilute specimen (water-loading): This may indicate dilution in some individuals. This does not necessarily represent a deliberate attempt at dilution. Caution must be exercised in interpreting low creatinine values as certain populations may have physiologically low levels. A repeat specimen is recommended.
< 0.44	Very Dilute	≤ 1.0010 or > 1.035	≥ 3 but < 4.5 or > 9.0 but < 11.0	< 10	Invalid specimen: The specimen characteristics are not consistent with human urine, or adulterated samples.
		> 1.035 and ≤ 1.040			The result may suggest possible contamination, very high levels of glucose, or recently received low-molecular-weight dextran or high-density radiopaque dyes. Also, could be suggestive of shock, nephrotic syndrome, dehydration, heart failure, liver failure, or acute glomerulonephritis.
			< 3.0 or > 11.0		Adulterated specimen: Is a specimen containing a substance that is not a normal constituent of urine or a specimen containing an endogenous substance not present at a normal physiological concentration.
		> 1.040			Adulterated specimen: The result may suggest the addition of salts or other adulterants to the urine sample received.

Table 4: *Creatinine cut-off values and relevant interpretation.*

**Creatinine cut-off values are based on the AS/ANZ 4308:2008 Standard.*

Discussion

An adulterated urine sample is defined as a specimen that contains a substance that is not endogenous in a normal physiological urine specimen. Specimen adulteration by using oxidising adulterants is not uncommon practice used to cheat a drug test. The aim is to produce

a false negative result by causing interference in the screening immunoassay methods. Using oxidising agents for adulteration oxidises drugs and/or drug metabolites to hinder drug detection either by screening or confirmation methods. When an oxidising agent is added to urine, it doesn't cause major changes to creatinine, SG or pH of the urine sample. Sometimes adulterated specimen is referred to as a "substituted specimen" which is not an accurate term to use as no assertion could be confirmed on a laboratory level that the specimen has been substituted. Therefore, the term "invalid specimen" is more accurate [7-9]. All samples identifies as "invalid specimens" should be retested using another aliquot of the urine specimen to ensure accuracy.

We found that 4.4% of the tested samples in our laboratory are flagged as synthetic urine. Also, we were able to break down the number of these flagged samples according to each clinic and project or estimate the possible numbers of synthetic urines based on the samples tested as shown in table 2. This information also can help different clinics tackle that issue and put in place a strict sample collection protocol to ensure specimen validity upon collection.

Methods measuring the temperature, pH, SG and creatinine concentration of the sample have been used to detect urine adulteration. The temperature of a freshly collected urine sample should be measured within 4 minutes of collection and if the temperature falls outside 32°C (90°F) and 38°C (100°F), it suggests sample tampering. The measurement of creatinine concentrations is an important variable for SVT and when attempting to determine if an individual has abstained from marijuana between successive urine specimens. Creatinine concentration reference intervals are 9.46 - 19.01 mmol/day (1070 - 2150 mg/day) in men and 6.75 - 10.61 mmol/day (764 - 1200 mg/day) in women [10]. Outside these ranges, the creatinine result should be interpreted alongside pH and SG to provide a proper interpretation of possible causes such as dilution or adulteration [7,9,11,12]. Excessive, fluid intake, a low protein diet and renal failure may cause creatinine concentration to fall below the reference interval. Normal urine creatinine is > 1.768 mmol/L and a dilute urine creatinine is ≥ 0.44 and < 1.768 mmol/L. One of the most common practices used to avoid a positive drug test is the intentional consumption of excess fluid in a relatively short period to flush the drug out. This is commonly referred to as water-loading or dilution and can result in the drug being below the positive cut-off concentration. If an individual produces urine with low creatinine concentrations, increasing the frequency of urine collections may not work because of the constant use of excess fluid. The donor should be instructed to limit their water intake to about half a litre/day on the day before testing and to donate the first urine morning sample for testing. This would minimise the likelihood of dilute urines.

The normal pH physiological range is 4.5 - 9.0. If pH is ≥ 3 and < 4.5 or > 9.5 and < 11, then the urine sample received is either adulterated or invalid (not consistent with a human urine sample). If pH is > 9 and < 9.5, this may be due to poor storage and/or Urinary Tract Infection (UTI) [7-9].

SG normal physiological range is 1.003 - 1.035 g/mL. If SG is < 1.003 g/mL, the result may suggest dilution of the urine sample received. If SG is > 1.035 and ≤ 1.040, the result may suggest possible contamination, very high levels of glucose, or recently received low-molecular-weight dextran or high-density radiopaque dyes. High SG is also seen in shock, nephrotic syndrome, dehydration, acute glomerulonephritis, heart failure, or liver failure. If SG is > 1.040, the result may suggest the addition of salts or other adulterants to the urine sample received.

The concentration of the carboxy-THC metabolite (Marijuana) can fluctuate from day to day depending upon a person's fluid intake. Increased fluid intake will lower both the carboxy-THC and creatinine concentration in a urine specimen while dehydration will have the opposite effect. Calculation of the carboxy-THC to creatinine ratio neutralises any change in the carboxy-THC concentration due to variation in a person's hydration state. This calculation makes it possible to compare urine specimens to establish if renewed use of marijuana has occurred. This ratio is the best indicator of continued drug use when compared to previous values on the same patient. Carboxy-THC can persist in the urine long after use from two days for the occasional user to six weeks for the chronic user. Metabolism rate and body fat are also factors in the length of time carboxy-THC may be detected in urine. Marijuana metabolite (carboxy-THC) when adulterated with

oxidants may produce a positive result when screened by immunoassay and interferes with the GC-MS confirmation method and gives a false negative result [13-15]. If drug adulteration alters the urine pH (acidity or alkalinity), it may facilitate faster drug elimination such as phencyclidine and amphetamines.

Some drugs and metabolites, such as morphine and 11-nor-Delta-9-Tetrahydrocannabinol-9-carboxylic acid, which is a metabolite of Delta-9-Tetrahydrocannabinol, may not be detected when an oxidising agent is added to the urine sample [15].

Reporting a false positive result due to some common medications, either prescribed or over-the-counter may affect someone's job application or terminate a career in professional sports [4]. Furthermore, the presence of some medical conditions may interfere with drug screening results [16].

A negative result may not inevitably mean that the patient is not using a drug. It may indicate that the patient drug use was under the cut-off value for the drug to be reported as positive or the method in use does not screen for the drug metabolites. For example, not all immunoassays detect fentanyl and its metabolites. When interpreting multiple results for methadone or buprenorphine, the pattern of urine results should be considered. A single positive urine result indicates recent drug use, while consecutive positive urine results may indicate either residual excretion or habitual drug use depending on the interpretation of the results [17].

It is also worth mentioning that many products sold on the internet to clear the drugs from the body and counteract drug detection are mostly diuretics which are medications designed to increase the amount of water and salt expelled from the body as urine. There are so many types of diuretics and some of them are from natural sources such as coffee, horsetail, parsley, hibiscus, caraway, green and black tea, *Nigella sativa* (black caraway, also known as black cumin) and dandelion extract (known as *Taraxacum officinale* or lion's tooth) [18].

The Department of Health and Human Services in the United States of America, and also Mayo Clinic Medical Laboratories have issued guidelines to address SVT, but no guidelines regarding synthetic urine or the assays evaluated in this paper [19,20]. SVT is essential in the screening process because it has a direct impact on results and earning confirmation testing. While SVT is not standardised, laboratories that should establish a robust SVT protocol to produce quality reliable results and assist with report interpretation.

Testing for general oxidants or specific oxidants, such as an oxidant assay, is valuable in providing further information regarding the validity of a urine sample. A positive result for oxidant activity should be considered when interpreting drug results. Oxidants can cause decreased levels or negative results for certain drugs, either by masking the drug's presence or by actually destroying the drug in the sample. The authors have published another study that compared the performance of the Sample Check assay and oxidant assay in the context of SVT [21].

Conclusion

In the case of synthetic urines, the Axiom assay detects four endogenous substances in urine and the outcome is interpreted alongside pH and SG results. The Axiom assay is recommended for testing with creatinine, pH, SG and oxidant assay, for screening both adulterated and synthetic urines. When urine samples are tested for sample integrity, if a sample is flagged because of this screening process, then this sample should be further tested by Axiom assay to detect synthetic urines. Also, the Axiom assay could be employed as one of the parameters tested in SVT. In addition, our laboratory is currently setting up an additional novel LC-MS/MS method for confirmation when a urine sample is flagged because of the above screening process.

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Conflict of Interest

The author declares that he has no conflict of interest.

Ethical Approval

This article does not contain any studies with human participants or animals performed by the author.

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