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RESEARCH ARTICLE

In Drugs of Abuse Sample Validity Testing, Which Assay is Telling the Truth? A Comparison Between Sample Check Assay and Oxidant Assay

Ashraf Mina[1,2*], Leah McNeice[1], Shanmugam Banukumar[1] and Santiago Vazquez[1]

[1]*NSW Health Pathology, Forensic & Analytical Science Service (FASS), Australia*

[2]*Affiliated Senior Clinical Lecturer, Faculty of Medicine and Health, Sydney University, Australia*

***Corresponding author:** *Dr. Ashraf Mina, NSW Health Pathology - Forensic & Analytical Science Service, PO Box 53, North Ryde Mail Centre, North Ryde NSW 1670, Australia, Tel: +61-02-9887-5666; +61-0403-108-682, Fax: +61-02-9805-1259*

Abstract

Background: Sample adulteration and synthetic or fake urines are challenges laboratories need to address when testing for Drugs of Abuse. Creatinine, pH, Specific Gravity (SG) assays are commonly tested for sample validity, but these assays are not sufficient to address sample adulteration and synthetic urines. Sample Check and Oxidant assays were evaluated in a study to examine which assay would add more value to sample validity testing.

Methods: We analysed 612 urine samples using creatinine assay, Sample Check assay and Oxidant assay which are analysed by using chemical methods on Beckman-Coulter chemistry analyser 5810. Also, the samples were tested with Drug Adulteration Test Strip from Teco Diagnostics.

Results: Out of the 612 specimens analysed, 7 specimens were reported positive using the Oxidant assay but were reported as normal using Sample Check assay. These 7 specimens were also tested using Urine Check 7 Drug Adulteration Test Strip and only one sample tested positive for nitrite. These 7 specimens were retested again after 13 days to examine the stability of the oxidants where only 4 of the 7 specimens tested positive. Oxidants may not be stable over time due to the breakdown of the oxidant material.

Conclusions: Oxidant assay is picking up a range of oxidants as adulterants efficiently, where some of these oxidants are more stable than others. Some oxidants are breaking down over time and become undetectable. Sample Check assay failed to pick up these oxidants. As a result, our laboratory addedthe Oxidant assay as part of sample validity testing and decommissioned Sample Check assay.

Keywords

Drugs of Abuse, DOA screening, Sample validity, Synthetic urine

List of Abbreviation

6-MAM: 6-monoacetylmorphine (heroin); CEDIA: Cloned Enzyme Donor Immunoassay; DRI: Diagnostic Reagents Inc; EIA: Enzyme Immunoassay; EMIT: Enzyme Multiplied Immunoassay Technique; N: Number of specimens; SD: Standard Deviation; SG: Specific Gravity

Introduction

Urine drug screening is a clinical tool that can enhance workplace safety, monitor patients' medication compliance, and detect illicit and prescription drug abuse as well as prescription medication diversion [1]. People use many methods to try to defeat human urine drug testing either by orally ingesting detoxification or flushing agents, diluting with water and other liquids and/or adulteration with other chemicals such as bleach, substitution with drug-free human urine or synthetic urine [2].

When assessing for medication adherence, a laboratory should be looking closely at the detection of an illicit substance and the possibility of a false-negative result due to adulteration or substitution of the tested sample. Adherence can be masked by many factors such as dilute urine, cleansing products, urine additives,

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the quantity of drug consumed, metabolism, time since the last dose, substituted urine sample, synthetic urine substitutes or the laboratory's assay cut-off level. Negative results in a dilute urine specimen may lead to misinterpretation of results. The Internet offers a wealth of information regarding techniques to pass a standard drug test. To counter these efforts, the Substance Abuse and Mental Health Services Administration (SAMHSA) in the United States of America (USA) mandate the testing of creatinine, SG, and pH on all urine samples to verify specimen validity [3]. The temperature of a sample should be taken within 4 minutes of collection, if possible, and should fall between 32 °C (90 °F) and 38 °C (100 °F). A urine temperature that falls outside of this range suggests tampering with that sample.

Each laboratory screening for drugs of abuse should establish a protocol in place for sample validity to address current challenges in this area. Measures can be put in place to reduce the chance of urine tampering. Our study investigated Sample Check assay and Oxidant assay to evaluate which assay would add more value to sample validity.

Materials and Methods

Beckman-Coulter AU5810 analyser was used for testing creatinine, Sample Check, Oxidant assays. Also, Techo Urine Adulteration Test strip 7 tests™ were used to test samples that tested positive by Oxidant assay and/or Sample Check assay.

The reagents obtained from Specialty Diagnostix and their part numbers are chromium (VI) validity calibrator (10445277), Oxidant perfect assay (10445267), UTAK validity control 2 (10445225), UTAK validity control 5 (10445228). The reagents obtained from Thermo Fisher and their part numbers are CEDIA Sample Check (CDF1815555), CEDIA Sample Check control (CDF1815571), Creatinine-detect (CDF1797), Creatinine calibrator set (CDF100272), and UrineCheck 7 Drug Adulteration Test Strip from Teco Diagnostics (CDA700-25). Techo Urine Adulteration Test strip 7 tests are used to detect Creatinine, pH, SG, nitrite, bleach, pyridinium chlorochromate, and glutaraldehyde.

The CEDIA Sample Check assay determines if a urine sample contains any compounds that may compromise the ability of the CEDIA assays for drugs of abuse screening such as detergents, bleach, vinegar, chromate, nitrite or goldenseal tea have been added to the urine samples by the illicit drug users before submitting their urine samples for the drug screening test. Successful adulterants can produce a false negative result for abused drugs by reducing the signal produced by immunoassays thereby avoiding detection. The CEDIA Sample Check assay uses recombinant DNA technology (US Patent No. 4708929) based on the bacterial enzyme β-galactosidase, which has been genetically engineered into two inactive fragments. These fragments spontaneously reassociate to form a fully active enzyme that, in the assay format, cleaves a substrate, generating a colour change that can be measured spectrophotometrically. In the assay, any compound that interferes with the ability of the fragments to reassociate and form active enzyme, affects the ability of the active enzyme to cleave substrate by denaturing the enzyme, blocking the active site, or preventing the colour change of the cleaved substrate by destroying either the substrate or the product, will be identified by a reduction in assay signal. The amount of signal reduction is dependent on the amount and composition of the interfering compound present. If the sample does not contain an interfering substance then the signal generation system will not be impacted and will fall within a normal range [4]. Oxidant assay can be performed on an automated clinical chemistry analyser to detect oxidants. The method is based on the reaction between the substrate Tetramethylbenzidine (TMB) and the oxidant in the sample producing colour that can be measured at 660 nm. Creatinine level was analysed for all samples. The method used is based on the Jaffe reaction, whereby creatinine concentration is determined colourimetrically using alkaline picrate to form a reddish Janovski complex [5].

Results

The study evaluated Sample Check and Oxidant assays using 612 urine samples. Creatinine and drugs of abuse were analysed for all samples and all positive results were confirmed by GC-MS or LC-MS. Out of the 612 specimens analysed, 7 specimens were reported positive using the Oxidant assay but were reported as normal using Sample Check assay. These 7 specimens were tested using Urine Check 7 Drug Adulteration Test Strip and only one sample tested positive for nitrite. These 7 specimens were retested again after 13 days to examine the stability of the oxidants where only 3 of the 7 specimens tested positive. Oxidants may not be stable over time due to the breakdown of the oxidant material (Table 1).

Using Oxidant assay, the following compounds at the stated concentrations yield a positive result relative to 50 µg/mL chromium (VI) cut-off, as indicated in the package insert. Sample check assay failed to identify these specimens tested positive for oxidants [6] (Table 2).

Some of these oxidants are less stable than others over time and may become undetectable. Therefore, early analysis of urine specimens is strongly recommended when Oxidant assay is used to check sample validity. The CEDIA Sample Check assay has been formulated to be more sensitive to specimen variation than other CEDIA assays. Because of the increased sensitivity to sample variation, the expected value range for a sample with no loss in integrity is 85-105%. Laboratories may narrow or expand their expected value range based on local sample population characteristics. Our laboratory

Table 1: Oxidant assay versus Sample Check assay, N = 612.

Sample ID	Oxidant Assay (Cut-off = 50.0)		Sample Check Assay (Reference Interval 88%-104%)		UrineCheck 7 Drug Adulteration Test Strip	
	Day 1	Day 13	Day 1	Day 13	Day 1	Day 13
Patient 1	**66 (Positive)**	1 (Negative)	Normal	Normal	Nil	Nil
Patient 2	**110 (Positive)**	**68 (Positive)**	Normal	Normal	Nil	Nil
Patient 3	**52 (Positive)**	40 Negative	Normal	Normal	Nil	Nil
Patient 4	**285 (Positive)**	**298 (Positive)**	Normal	Normal	**Positive for Nitrite**	**Positive for Nitrite**
Patient 5	**64 (Positive)**	48 (Negative)	Normal	Normal	Nil	Nil
Patient 6	**76 (Positive)**	50 (Negative)	Normal	Normal	Nil	Nil
Patient 7	**73 (Positive)**	**75 (Positive)**	Normal	Normal	Nil	Nil

Urine Check 7 Drug Adulteration Test Strip was used for positive samples.

Table 2: Oxidising agents at the stated concentrations that yield a positive result relative to a 50 µg/mL Chromium (VI) cut-off by Oxidant Assay.

Pyridinium Chlorochromate	220 µg/mL
Bleach	11 mg/dL
Nitrite	40 µg/mL
Iodine	65 mg/dL
Periodate	90 mg/dL
Peroxidase/Peroxide	0.30%
Iodate	750 mg/dL
Iodic Acid	600 mg/dL

analysed 2015 urine samples to establish our reference intervals (88% to 104%). If the assay result is ≥ 88 to ≤ 104, the specimen is considered normal. If the result is < 88 or > 104, the specimen is considered abnormal and should be further tested by UrineCheck 7 Drug Adulteration Test Strip. The result should be interpreted based on the outcome of the 7 tests and the creatinine result together.

There are some limitations of the Oxidant assay; if urine specimens from individuals who take herbal supplements containing concentrated cranberry extract, an oxidant, may test positive and also if ascorbic acid is present, an antioxidant, may interfere with the Oxidant assay. Some oxidising agents are not stable in urine and their levels may diminish over time.

Discussion

Specimen Validity Testing (SVT) is an important part of every urine drug test. It provides clinicians with critical information about the accuracy and reliability of drug test results, and that the specimen submitted is a valid human urine specimen. Also, it will assist regarding potential drug abuse, mismanagement of medications, or diversion of prescribed drugs. A complete urine drug of abuse testing program normally involves specimen collection, initial screening with an immunoassay, followed by a confirmation test, such as Liquid Chromatography-Mass Spectrometry (LC-MS) or Gas Chromatography/Mass Spectrometry (GC/MS), for the positive samples [7].

A person could be affected by a false positive when applying for a job or playing professional sports due to some common medications, either prescribed or over-the-counter. Moreover, the presence of some underlying medical conditions may obscure the urine drug screening results [8]. The use of oxidising adulterants is one of the most common ways donors try to cheat a drug test. Successful adulterants can produce a false negative result for abuse drugs by reducing the signal produced by immunoassays thereby avoiding detection. An adulterated specimen is a urine 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. An oxidising adulterant is a substance that acts alone or in combination with other substances to oxidise drugs or drug metabolites to prevent the detection of the drugs or drug metabolites, or affects the assays in either the initial or confirmatory drug test.Sometimes such a sample is referred to as a “substituted specimen”. This term is not accurate as no assertion could be

confirmed on a laboratory level that the specimen has been substituted. Therefore, the term "invalid sample" is more accurate [9-11]. All "invalid" specimens," based on analytical test results, should be retested on another aliquot of the urine specimen to ensure accuracy. All "invalid specimens", based on analytical test results, should be retested on a different aliquot of the urine specimen to ensure accuracy. Adulteration methods include dilution with water, substitution with a drug-free liquid, the addition of readily available household materials (e.g., vinegar, baking soda, liquid drain opener, detergent, etc.). Several oxidising adulterants are being sold with a claim to clear all positive drug test results. The most commonly used oxidising adulterants are nitrite (Klear™), chromate (Urine Luck™), iodine, bleach and horseradish peroxidase/H_2O_2 (Stealth™). When added to urine, there is no significant change to the appearance, pH, specific gravity or creatinine concentration.

Marijuana samples adulterated with oxidants may produce a positive result, during an initial screening by immunoassay, notably the marijuana metabolite (THC). However, they can not be confirmed by GC/MS [12,13]. Drug users may alter their urine pH (acidity or alkalinity) to facilitate faster drug (e.g., phencyclidine, amphetamines) elimination.

Creatinine is found naturally in the urine. It is produced by the breakdown of muscle tissue and cleared from the body via the kidneys. A creatinine level outside of reference values may result from excessive fluid intake, renal failure, diet, or many other medical conditions or factors. If the specimen results in a creatinine > 1.7680 mmol/L (0.02 mg/dL) and a positive Sample Check result, it will be reported as "normal creatinine with 'positive' Sample Check". If the specimen results in a creatinine < 1.7680 mmol/L (0.02 mg/dL) and a positive Sample Check result, it will be reported as "low creatinine and positive Sample Check".

The temperature of a sample should be taken within 4 minutes of collection, if possible, and a urine temperature that falls outside of this range suggests tampering with that sample. It is recommended that urine drug collection cups with integrated temperature measuring strip should be used and urine temperature recorded after the sample collection and notified for the laboratory if it falls between 32 °C (90 °F) and 38 °C (100 °F).

Some drug analytes, in particular morphine and 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid, a metabolite of delta-9-tetrahydrocannabinol, could not be detected in the presence of some oxidising agents [14].

The U.S.A Department of Health and Human Services and also the Mayo Clinic Medical Laboratories has issued guidelines to address the sample validity issue, but no guidelines regarding synthetic urine or the assays evaluated in this paper [15,16]. 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.

Conclusion

Oxidant assay is picking up a range of oxidants as adulterants efficiently, where some of these oxidants are more stable than others. Some oxidants are breaking down over time and become undetectable. Sample Check assay failed to pick up these oxidants. As a result, our laboratory added the Oxidant assay as part of sample validity testing and decommissioned Sample Check assay.

Future Direction

When urine samples are tested for sample integrity using creatinine, pH, specific gravity and Oxidant assays, if a sample result is flaggedas a result of this screening process, then the sample will be further tested by another novel assay designed to detect synthetic urines. Our laboratory is currently evaluating this novel assay and we will publish the outcome.

Compliance with Ethical Standards

- **Funding:** No funds were received for this article.

- **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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