Technical Note

Effects of Stealth™ Adulterant on Immunoassay Testing for Drugs of Abuse*

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Abstract

Stealth is an adulterant advertised as being undetectable by adulteration tests. It has been described as peroxidase and peroxide, which, when added to urine samples, are intended to prevent a positive drug test. Characterization of the effect of Stealth on urine samples and immunoassay results was undertaken to assist in detection of this adulterant. Stealth was added to a number of urine matrices, and various parameters were evaluated including pH, specific gravity, color, creatinine, chloride, urea, blood, glucose, and nitrite. Samples were spiked with THC acid metabolite, benzoylmorphine, morphine, secobarbital, PCP, amphetamine, and lysergic acid diethylamide (LSD) then tested by Roche OnLine and Microgenics CEDIA immunoassay reagents. Results of these analyses showed Stealth did not cause the urine sample to exceed any of the monitored parameters including those routinely used in drug-testing laboratories that would indicate adulteration of a sample. It did, however, cause samples positive for the marijuana metabolite (11-nor-Δ⁹-tetrahydrocannabinol-9-carboxylic acid), LSD, and opiates (morphine) at 125–150% of cutoff to screen negative by immunoassay. Adulterating an authentic positive sample provided by a marijuana user caused that sample to screen negative using these immunoassay reagents as well.

Introduction

The use of adulterants to avoid detection of drug abuse has a long history. Initially, adulteration of urine samples was accomplished using readily available materials (soap, detergent, household cleaners, etc.). Although some of these were effective in interfering with the testing procedure, many could easily be detected by smell or appearance changes to the sample (1–4). As drug-testing programs have become more sophisticated, so too have the procedures used to adulterate samples. An industry to provide drug abusers with the means to adulterate samples with the sole purpose of avoiding detection of their illicit drug use has developed. These adulterants are more sophisticated in that they are developed specifically for the purpose of adulterating urine samples and are designed, as much as possible, to avoid detection. Some examples of these products include the fixative glutaraldehyde (UrinAid and Clear Choice), strong inorganic acid (Amber-13 and THC-Free), and strong oxidants such as nitrite (Klear, Whizzies, and Randy’s Klear) and chromate (Urune Luck, LL 418, Sweet Pee’s Spoiler, and Randy’s Klear II) (2,5–14).

Recent procedures placed in effect by drug-testing programs have lead to the identification of an ingredient of these adulterants, and laboratory tests have been developed to detect their presence. These tests range from simple measurement of pH to specific tests for the adulterant itself. Many of these adulterants can now be easily detected and their presence confirmed. Recent directives from the Substance Abuse and Mental Health Services Administration (SAMHSA) have defined procedures for the identification and reporting of adulterated samples (15,16). The new policy places significant weight on the use of an adulterant to avoid detection of illicit drug use.

One report indicated that Stealth was available to adulterate urine samples and that it caused the screening assay for the THC acid metabolite (11-nor-Δ⁹-tetrahydrocannabinol-9-carboxylic acid, THCCOOH) to yield negative results. The product is provided as two vials: one containing peroxidase and the other containing peroxide (17). Combination of these two components would provide strong oxidation potential in a urine sample. Peroxidase activity in urine samples can be detected using a number of commercially available peroxidase assays, but these are not routinely used to assess samples for adulter-
The current study was designed to assess the effect of the adulterant on various clinical parameters and immunoassay testing for drugs of abuse.

Materials and Methods

Materials
Drugs were obtained from the following sources: Sigma (amphetamine, phencyclidine [PCP], morphine, morphine-3-glucuronide, lysergic acid diethylamide [LSD], secobarbital, THCCOOH), Alltech (benzoylecgonine, LSD, PCP), Radian (secobarbital, LSD), and Research Triangle Institute (THC-COOH). (Note that drugs from multiple manufacturers were obtained to allow calibrators and controls to be prepared from different source material.) Immunoassay reagents used in this study for THC metabolite, cocaine metabolite, opiates, barbiturates, PCP, and amphetamines were OnLine reagents from Roche Diagnostics and CEDIA from Microgenics. LSD immunoassay reagents used were EMIT II from Behring and CEDIA from Microgenics. Fresh human urine was collected to prepare spiked samples, and a sample from a marijuana user, previously confirmed positive for THCCOOH, was used for evaluation. Random urine samples were also collected for evaluation of the effect of Stealth on clinical parameters such as pH, specific gravity, and creatinine. Clinical dipsticks (Multistix) used for testing the samples were from Bayer Corp. Tetramethylbenzidine (TMB) substrate reagent set for detection of peroxidase activity was from Pharmingen, and horseradish peroxidase was obtained from Sigma. nT PERFECT was obtained from Chimera Research and Chemical. Stealth was obtained from the supplier and provided to these investigators by the Research Triangle Institute and the Air Force Office of Special Investigation.

Methods
Fresh urine (no preservatives) was split into different portions for use in evaluation of the adulterant. Initially, the sample was split into two portions, one of which was used as negative control material, and the other was spiked with 67.5 ng/mL of the THC acid metabolite (135% of cutoff), 187.5 ng/mL of cocaine metabolite benzoylecgonine (125% of cutoff), 2500 ng/mL of morphine (125% of cutoff), 250 ng/mL of secobarbital (125% of cutoff), 31.25 ng/mL of PCP (125% of cutoff), 625 ng/mL of amphetamine (125% of cutoff), and 750 pg/mL of LSD (150% of cutoff). Additionally, other samples were spiked with 60 ng/mL of THC-COOH, 800 pg/mL of LSD, and 6000 ng/mL of morphine glucuronide and codeine (The latter concentrations were equivalent to median concentrations seen for these drugs in data collected for the previous year). The spiked samples

Figure 1. Effects of Stealth adulterant on OnLine immunoassay. Results from Roche OnLine immunoassay analysis of samples spiked with drug or drug metabolite six hours following adulteration. Results are normalized for comparison by using the average response (n = 3) of the positive control as 100% and plotting the average response of the adulterated aliquot as a percentage of the positive control. Amp = amphetamine (625 ng/mL), Barb = secobarbital (250 ng/mL), Cocaine = benzoylecgonine (187.5 ng/mL), PCP = PCP (31.25 ng/mL), Op-2500 = morphine (2500 ng/mL), Op-6000 = morphine glucuronide (6000 ng/mL) and codeine (6000 ng/mL), THC-1 = THC acid metabolite (67.5 ng/mL), THC-Real = sample from marijuana user, LSD-1 = LSD (750 pg/mL) and LSD-2 = LSD (800 pg/mL).
were split and Stealth added to one portion following package directions. The samples were then aliquoted and assayed in replicate for each of the drug classes. The effect of time was monitored by testing samples at 6, 24, 48, and 72 h and 7, 13, and 21 days following adulteration using OnLine reagents. Mean responses from the replicates \((n = 3)\) were then plotted with the adulterated aliquot results reported as a percentage of the unadulterated positive control readings. Analysis of these samples was also accomplished using CEDIA immunoassay reagents.

Immunoassays were performed on Olympus AU800 and Hitachi 911 or 902 analyzers using parameters defined by the reagent manufacturers for the respective instruments. Cutoffs followed the current Department of Defense immunoassay cutoff levels for drugs of abuse: 50 ng/mL for THC metabolite, 150 ng/mL for cocaine metabolite, 2000 ng/mL for opiates, 200 ng/mL for barbiturates, 25 ng/mL for PCP, 500 ng/mL for amphetamines, and 500 pg/mL for LSD.

A simple and quick color test to detect the presence of Stealth in a urine sample was made using commercially available reagents (TMB substrate reagent set, Pharmingen) for detection of peroxidase. The test was performed by adding 10 μL of urine to a test tube containing 50 μL of TMB substrate reagent in 500 μL of 0.1M phosphate buffer (pH 7). The sample was mixed and observed for an immediate color change.

Results and Discussion

Results of immunoassay analysis of sample aliquots using the Roche OnLine reagents spiked with cocaine metabolite (benzoylcegonine), barbiturates (secobarbital), PCP, and amphetamines (amphetamine) were not affected by adulteration with Stealth (Figure 1). Comparison of the unadulterated and adulterated sample aliquots showed no discernible difference before and after treatment. The results remained consistent when tested at 6, 24, 48, and 72 h and 7, 13, and 21 days following adulteration. Results using the CEDIA assays are shown in Figure 2 and were comparable to those seen with the OnLine assay.

The effect on the Roche OnLine opiate assay was substantial, yielding a negative result for the sample containing 2500 ng/mL morphine. Unlike the THC and LSD assay results, the readings of the adulterated opiate samples were approximately 30% of the control positive throughout the time period covered by the study. The consistent response of the assay led to the thought that the effect was concentration dependent. This was further investigated using samples spiked with 6000 ng/mL of both codeine and morphine glucuronide. These samples were also adulterated with Stealth and analyzed by immunoassay. In this case, both the control positive and the adulterated aliquot tested positive by both assays. The readings of these high concentration samples, which were run undiluted, were essen-

![Figure 2](image-url)

**Figure 2.** Effects of Stealth adulterant on CEDIA immunoassay. Results from Microgenics CEDIA immunoassay analysis of samples spiked with drug or drug metabolite. Results are normalized for comparison by using the average response \((n = 3)\) of the positive control as 100% and plotting the average response of the adulterated aliquot as a percentage of the positive control. Amp = amphetamine (625 ng/mL), Barb = secobarbital (250 ng/mL), Cocaine = benzoylcegonine (187.5 ng/mL), PCP = PCP (31.25 ng/mL), Op-2500 = morphine (2500 ng/mL), Op-6000 = morphine glucuronide (6000 ng/mL) and codeine (6000 ng/mL), THC-1 = THC acid metabolite (67.5 ng/mL), THC-2 = THC acid metabolite (60 ng/mL), THC-Real = sample from marijuana user, LSD-1 = LSD (750 pg/mL) and LSD-2 = LSD (800 pg/mL).
tially the same, presumably because the concentrations of both were far above the linear range of the assays. The results also indicate the effect of Stealth on the immunoassay of opiates is concentration dependent because even a low concentration of the drug (25% above cutoff) showed some measurable activity, albeit reportable as 'negative'. No further experiments were conducted to determine the exact amount of opiate that could be present and still result in a negative reading on immunoassay. These results suggest that many Stealth-adulterated samples positive for opiates will still screen positive by immunoassay. Attempts using standard assay procedures to confirm the presence of opiates by gas chromatography–mass spectrometry (GC–MS) failed to recover the drugs or their respective internal standards in several of these Stealth-adulterated samples.

The THC assay showed the adulterated aliquots of spiked samples to give clearly negative results using the OnLine (Figure 1) and CEDIA reagents (Figure 2). It is well known that there are many metabolites of THC found in urine samples following marijuana use, and the potential exists for one or more of these metabolites to not be affected by Stealth and cause the immunoassay results to be positive despite the adulteration. This possibility was evaluated by taking a sample that had previously tested positive by immunoassay and GC–MS for marijuana. This sample was taken from frozen storage and split into two portions, one of which was adulterated with Stealth. Aliquots of the adulterated and unadulterated samples were then analyzed by both immunoassay procedures. Results showed the unadulterated aliquots were positive, but the Stealth-adulterated aliquots gave negative results with both immunoassays (Figures 1 and 2). Analysis of this sample by GC–MS showed 52 ng/mL THCCOOH following removal from frozen storage, a value comparable to the 56 ng/mL determined on initial analysis. However, THCCOOH could not be detected in the corresponding Stealth-adulterated aliquots by GC–MS. This result indicates samples provided by marijuana users and adulterated with Stealth will likely be affected when tested by these immunoassay reagents and GC–MS. Even had these immunoassays tested positive, the sample would not confirm positive by GC–MS as no THCCOOH was detected in the Stealth-adulterated samples. As seen with the THC assay, aliquots of sample spiked with LSD and adulterated with Stealth were negative using EMIT II (Figure 1) and CEDIA reagents (Figure 2).

Evaluation of the urine samples following addition of Stealth revealed no changes in parameters normally used to assess adulteration that would raise suspicion about the sample. Creatinine, specific gravity, and pH were measured before and after addition of Stealth, as were observations of the sample smell and color. In every case, the urine appeared slightly darker after the addition of Stealth, but not to the extent this change in color would raise suspicion of adulteration. The effect on pH varied dramatically between samples and ranged from small to fairly large changes; however, none of the samples were outside the acceptable (15) pH range of 3–11 following adulteration with Stealth (Table I). The higher the initial pH of the sample, the greater the change. Most samples showed a small but unremarkable change in specific gravity and creatinine (Table I). Values for chloride and urea also showed no differences that would lead to characterization of the sample as being adulterated.

Use of a clinical dipstick showed all samples adulterated with Stealth gave strong positive readings for glucose, blood, and nitrite. The reaction with the dipstick appears related to the fundamental principles of the impregnated tests for these analytes. The glucose reaction on the test strip is a peroxidase-based coupled reaction. The test for blood is also based on peroxidase activity (based on the pseudoperoxidase activity of the heme), and the nitrite test is based on the reaction of para-arsanilic acid with nitrite followed by formation of the diazonium complex. Any existing urinary nitrate could be converted to nitrite by the redox reaction due to Stealth. It is also possible for other urine constituents with sufficient redox capacity, imparted by reaction with the Stealth, to interact with these reagents giving rise to a positive result. Except for nitrite, these analytes are not routinely used to assess adulteration of urine samples. None of the urine samples gave a positive reaction to these parameters prior to the addition of Stealth. This triad of positive dipstick results was seen for all adulterated samples and is an unusual clinical finding. These results represent a significant change in response for all of these samples ($p < 0.01$; sign test). One of the samples adulterated with Stealth was monitored for these parameters for 21 days. All three parameters remained positive during that period. The nitrite response, although still positive, appeared weaker (less color change) as time progressed. Analysis of Stealth-adulterated samples using nT PERFECT (a specific test for nitrite) did not result in a positive nitrite test. Remembering that the clinical dipsticks are designed to identify clinically relevant levels of nitrite that are far below the cutoff level of nitrite required to report a sample as adulterated, these results should not be considered contradictory.

Using the commercially available peroxidase assay reagents described earlier, Stealth-adulterated samples exhibited a

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<th>Table I. Effect of Stealth Adulterant on Urine Specific Gravity, Creatinine, and pH</th>
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rapid color change from clear to dark brown. The potential for interference from other compounds (i.e., hemoglobin, bacteria, etc.) that may also show peroxidase activity was assessed. Urine samples (n = 167) from the clinical laboratory that had previously tested positive for glucose, nitrite, and/or blood were tested using this assay. Most of these samples showed no color development at all. Several did develop some color (blue-green); however, this was easily distinguished from the dark brown color seen with Stealth-adulterated samples. The color test provides a quick and inexpensive way to detect peroxidase activity if Stealth is a suspected adulterant. This qualitative test can also be adapted for use with a spectrophotometer or autoanalyzer.

Conclusions

Addition of Stealth to urine samples does not result in a change that would be detected by current routine analyses designed to identify adulterated specimens. The results of Roche OnLine and Microgenics CEDIA immunoassay analysis of cocaine metabolite (benzylecgonine), barbiturates (secobarbital), PCP, and amphetamines were not affected by this adulterant. Results of spiked THC metabolite and LSD were significant and were clearly negative with both assays. Adulteration of an actual specimen from a marijuana user also caused a negative result using OnLine and Microgenics CEDIA reagents. The response to the opiate assay was decreased demonstrating the possibility for low concentration samples to drop below cutoff. High-concentration opiate samples, however, still gave positive immunoassay results.

Acknowledgments

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References