

# Toxicology News

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## Adulterants can Interfere with Urine Drugs-of-Abuse Tests

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**I**n the U.S. military drug-testing program and others in which urine collections are observed, test subjects have little opportunity to adulterate their urine specimens. However, when direct observation of specimen collection is not routine, as is the case in most employment testing programs, some subjects attempt to subvert their tests by adulterating their specimens.

### Common household chemicals as adulterants

The primary goal of specimen adulteration is to produce a negative result in a specimen in which drug is present. The ideal adulterant would produce a negative result on the initial test and be undetectable by the laboratory. A variety of additives have been utilized in that regard, with mixed results.

Examples of common household products reportedly used as adulterants include table salt, vinegar, liquid laundry bleach, concentrated lemon juice, golden seal tea (produces dark urine), and Visine eye drops. Some of these products may be effective, depending largely on the assay used.

One early study assessed the effects of various adulterants on radioimmunoassay (RIA), fluorescence polarization immunoassay (FPIA), and enzyme-multiplied immunoassay test (EMIT) for amphetamines, barbiturates, benzodiazepines, marijuana (cannabinoids), cocaine, opiates, and PCP (1). Sodium chloride caused negative interference with all drugs tested by EMIT and caused a slight decrease in measured concentrations of benzodiazepines by FPIA, but did not affect RIA results. Bleach decreased results for most drugs by all three methods, but had a positive effect on the RIA barbiturate test and on the FPIA benzodiazepine test in a sample containing no drug. Sodium bicarbonate caused false-positive results with the EMIT opiates assay

and with the FPIA PCP assay. Hydrogen peroxide caused false-positive benzodiazepine results by FPIA.

Uebel and Wium studied the effects of sodium hypochlorite, the antiseptic Dettol (chloroxylenol), glutaraldehyde, Perle hand soap, ethanol, isopropanol, and peroxide on cannabinoids and methaqualone tests using EMIT assays (2). Most of the agents interfered with the tests. The greatest effect was observed with glutaraldehyde and Perle hand soap causing false-negative results in methaqualone testing. Dettol and Perle hand soap also caused false-negative results in cannabinoids tests. The addition of isopropanol, ethanol, and peroxide invalidated the methaqualone test.

### Collection site deterrence

The collection site and the laboratory can use a number of mechanisms to deter and detect adulterated specimens. Many collection sites and laboratories comply with the procedures required for federal workplace drug-testing programs developed by the Department of Health and Human Services (DHHS) and the Department of Transportation (DOT) (3, 4).

Both DHHS and DOT have standardized collection procedures that address the detection and prevention of specimen adulteration at the collection site. DHHS requires laboratories to perform "specimen validity tests" for all federal employee specimens. At this time, transportation industry employers subject to the DOT regulations may, but are not required to, perform specimen validity testing. If they do so, the testing must comply with the DHHS guidelines. Many private-sector employers use the

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## Crystal Meth: Abuse, Toxicity And Laboratory Detection

By Ping Wang, Christine A. Haller, and Alan H.B. Wu

*“For a while, deep down underneath it all, you still know you got that dread that this high is going to wear off. That’s why you don’t stop doing it. Because as soon as you stop doing it, you’ll have to deal with that horrible despair and depression. The way to avoid that is, you just don’t come down (1).”*

The above paragraph describes the typical experience of drug abusers indulging in crystal methamphetamine (crystal meth). An insidious recreational drug, methamphetamine was first synthesized in Japan in 1893, where it was widely distributed in the armed forces as a stimulant during World War II. After the war, a massive supply of methamphetamine unused by the military became available to the public in Japan, leading to widespread abuse.

In the 1950s and 1960s, methamphetamine was prescribed in the United States to treat obesity, depression, narcolepsy, alcoholism, and even post-encephalitic parkinsonism. The recreational use and clandestine production of methamphetamine in the United States rose quickly afterwards, especially in the West, Midwest, and South. It is classified as a Schedule II substance under the Controlled Substances Act.

Today an estimated 4.7 million people, approximately 2.1% of the U.S. population, have used methamphetamine at least once in their lives (2). It is especially popular among the gay community, at gay clubs and circuit parties, where people claim to use crystal meth to escape loneliness and alienation, deal with aging and illness, and lower sexual inhibitions. Tragically, the abusers often have a hard time dealing with reality when they wake up from the “high” and are driven to take more crystal meth next time to

“fix” whatever is wrong with their lives—thus, a vicious cycle of abuse ensues. In addition to suffering from the toxicity of methamphetamine itself, abusers tend to withdraw from society, and frequent unsafe sexual behaviors lead to higher risks of sexually transmitted diseases, such as HIV and syphilis (3).

### Chemistry, CNS effects, and metabolism

Methamphetamine (R,S-N-methyl-1-phenyl-2-propanamine) is a chemically synthesized stimulant pharmaceutically referred to as methylamphetamine or deoxyephedrine. Its chemical structure is shown in Figure 1. Its numerous slang names include crystal, ice, speed, and working man’s cocaine. The purest form available illicitly is a colorless crystalline solid (hence the name of crystal meth). With decreased purity, it can be brownish rocks or powder. Methamphetamine is lipophilic, so it can cross the blood-brain barrier and penetrate neuronal plasma membranes (4). Table 1 lists its physical and chemical properties.

Methamphetamine can also enter neurons through the dopamine transporter on the presynaptic membrane. In neuronal cells, methamphetamine displaces neurotransmitters such as norepinephrine, dopamine, and serotonin from their vesicle stores into cytoplasm. It also causes the dopamine transporter to work in the reverse direction, pumping out neurotransmitters into the synaptic cleft. In addition, methamphetamine inhibits monoamine oxidase B, the enzyme responsible for destruction of released neurotransmitters. The combined effects are massively increased norepinephrine, dopamine, and serotonin concentrations in the central nervous system. Users usually have feelings of enhanced well-being, increased energy, heightened libido, and suppressed appetite. High doses can cause euphoria, paranoia, agitation, and psychosis.

Methamphetamine provides longer-lasting stimulant effects than cocaine due to a longer half

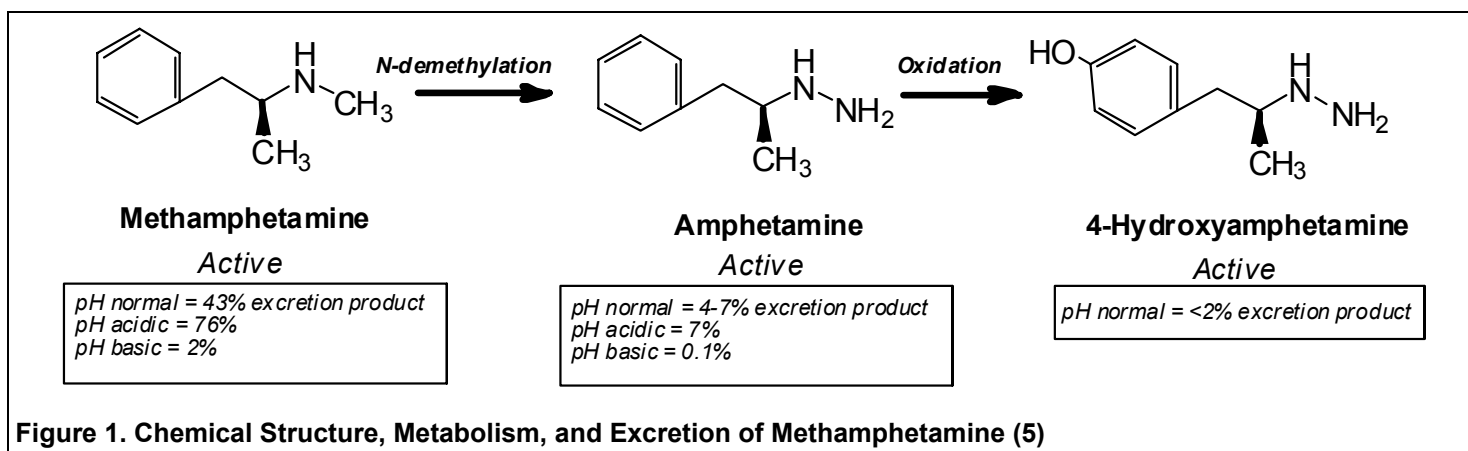


Figure 1. Chemical Structure, Metabolism, and Excretion of Methamphetamine (5)

life (4–12 hours). Table 1 lists methamphetamine's pharmacokinetic parameters. At normal urine pH, about 43% of the dose is excreted unchanged in the urine, 4–7% undergoes N-demethylation to amphetamine, and less than 2% is converted to 4-hydroxyamphetamine. Other metabolism includes deamination and conjugation. The rate of methamphetamine excretion decreases when urine pH is more alkaline. Cytochrome P450 2D6 (CYP2D6) has been shown to be involved in both N-demethylation and aromatic 4-hydroxylation of methamphetamine (6, 7). Figure 1 summarizes methamphetamine metabolism and excretion.

### Pharmacogenomics

Epidemiology studies in twins suggest the heritability of psychostimulant abuse is as high as 66%, indicating underlying genetic factors (8). If genes that contribute to methamphetamine addiction can be identified, they may provide targets for therapeutic intervention. Genes that have been studied in relation to methamphetamine dependence include those encoding neurotransmitter metabolizing enzymes, such as catechol-*O*-methyltransferase (COMT) and dopamine beta hydroxylase (DBH).

Neurotransmitter receptors and methamphetamine-metabolizing enzymes (such as dopamine D2, D3, and D4 receptors; GABA A receptor gamma 2; and glutathione-S-transferase P1) have also been studied. Some researchers suggest that polymorphisms associated with decreased COMT activity may protect against methamphetamine abuse; others speculate that the 7-repeat polymorphism in exon III of dopamine D4 receptor is associated with methamphetamine abuse. However, results that contradict the

above suggestions have also been reported.

The I105V polymorphism of glutathione-S-transferase P1, which results in a 30% decrease in enzyme activity, is associated with psychosis but not spontaneous relapse in Japanese methamphetamine abusers. Clinical studies with greater statistical power are needed to further investigate the role of pharmacogenomics in crystal methamphetamine abuse.

### Clandestine synthesis

Crystal meth is much cheaper than drugs such as cocaine and heroin, which must be smuggled, because it can be synthesized easily from the non-prescription drugs ephedrine or pseudoephedrine and other readily available household products. Nonetheless, methamphetamine production in untrained clandestine labs is dangerous, leading to fires and explosions, which often reveal the locations of the labs. In 2004, there were 17,033 such incidents in the United States, according to the National Clandestine Laboratory Database (10). Although most of the crystal meth on the market today comes from large labs in California and Mexico, small-scale meth labs are distributed all over the country.

On March 9, 2006, the Combat Methamphetamine Act was enacted to provide minimum standards for national retailers that sell products containing ephedrine and pseudoephedrine. This law limits purchases to 3.6 grams of the pure base ingredient per day and 9.0 grams per 30 days. All buyers must provide identification and sign a sales log. In addition, sellers must limit access to these products to behind the counter or locked lo-

**Table 1. Physical and Chemical Properties and Pharmacokinetics of Methamphetamine (5, 9)**

Physical state: Colorless crystalline solid (purest form) to brownish rocks or powder
Molecular formula: C <sub>10</sub> H <sub>15</sub> N
Molecular weight: 149.24
pKa: 9.9
Solubility: Limited in water (lipophilic)
Elimination half-life (pH dependent): 10 h (range: 6–15) (oral); 12.2 h (intravenous); 11.1 h (smoked)
Volume of distribution: 3.0–7.0 L/kg
Plasma protein binding: 10–20%
Bioavailability: 67% (oral); 90% (estimated smoked dose)
Duration of action: 4 h to more than 12 h
Peak plasma concentration and time after oral administration:
0.02 mg/L at 3.6 h (0.125 mg/kg; 8.75 mg/70 kg dose)
0.03 mg/L at 1 h (0.143 mg/kg; 10 mg/70 kg dose)
0.032 mg/L at 7.5 h (0.286 mg/kg; 20 mg/70 kg sustained release dose)
0.094 mg/L at 7.5 h (0.428 mg/kg; 30 mg/70 kg sustained release dose)
Peak plasma concentration and time after smoking administration:
1.01 mg/L at 18 min (0.311 mg/kg; 21.8 mg/70 kg dose)
Peak plasma concentration and time after intravenous administration:
0.78 mg/L at 17 min (0.221 mg/kg; 15.5 mg/70 kg dose)

cations and register on-line with the U.S. attorney general.

Most methamphetamine synthesis procedures involve reduction of the hydroxyl group of ephedrine or pseudoephedrine. This is usually achieved by mixing ephedrine or pseudoephedrine extracted from over-the-counter medications with hydroiodic acid and red phosphorus, followed by heating for 24 hours. Highly toxic phosphine gas can be generated in this process, and manufacturers unaware of the danger may inhale it. Another variation uses metallic lithium from batteries and liquid anhydrous ammonium, which are both very reactive and can lead to explosions.

There are also environmental concerns, because clandestine manufacturers dump around six pounds of toxic waste into the environment for every pound of methamphetamine produced. Children in these "meth households" are especially susceptible to the toxicity of meth manufacturing, and are also at risk of neglect and abuse.

### Toxicity

Medical complications in many body systems can arise from methamphetamine abuse, including neurological, cardiac, pulmonary, and dental inju-

ries. Thirteen states reported hospital admission rates of more than 90 per 100,000 people of the general population for methamphetamine abuse and dependence treatment in 2002 (10).

The most well-documented toxicity of methamphetamine is the long-term damage to dopamine and serotonin nerve terminals. Neuroimaging studies of chronic abusers demonstrate significant losses in dopamine transporter availability in the brain (11). The reduction in dopamine transporters does not completely reverse even after methamphetamine use stops, and is evident as long as 14 months after last use. Loss of dopamine transporters is associated with reduced motor speed and impaired verbal learning. Dopamine D2 receptor is also severely down-regulated in methamphetamine abusers.

The neurotoxicity of methamphetamine is thought to be due to the large amount of reactive oxygen species resulting from elevated extracellular monoamines and glutamate, as well as methamphetamine-induced hyperthermia, ultimately leading to neuronal apoptosis (12). The S-enantiomer of methamphetamine is five times stronger in psychostimulant activity than the R-enantiomer.

Prolonged sympathetic nervous system stimulation from methamphetamine use increases myocar-

## Case Report: Methamphetamine Overdose

A 43-year-old male patient presented to the emergency department after a methamphetamine binge. Within two hours of the binge, he was found unresponsive by his partner. The patient had a core body temperature of 39.6°C, a systolic pressure of 70 mm Hg, and a heart rate of 150–160 beats/min. He was combative and agitated, and attempted to bite hands and feet. He had a rash-like skin erythema on his shoulder, neck, and fingers. He was noted to have "lead pipe muscle rigidity."

The patient received 5 liters of intravenous fluids and his blood pressure returned to normal. Cooling was initiated with wet blankets and ice packs. His stiffness abated after administration of anesthetics, sedatives, and neuromuscular blocking medications, but worsened as these medications wore off.

Urine toxicology screening by immunoassay was positive for amphetamine/methamphetamine. In confirmation testing with high performance liquid chromatography with ultraviolet detection, methamphetamine, without amphetamine, was found in the urine, consistent with a recent ingestion. His creatine kinase level was elevated at 200

μ/L, and subsequently rose to 900 μ/L. Blood cultures, head CT scan, and lumbar puncture studies were all negative. Serum electrolytes and the anion gap were normal.

In addition to acute methamphetamine toxicity, the differential diagnosis initially included Stevens-Johnson syndrome because of the presence of skin erythema and muscle rigidity. However, on follow-up, it was noticed that there was no vesicular-erythematous continuity in compressed skin folds, indicating the skin erythema was most likely caused by fume and/or vapor scalding during methamphetamine smoking. Tetanus was also considered in the differential diagnosis because of the muscle rigidity, but was ruled out by the rapid onset of symptoms. The muscle rigidity was thought to be due to exacerbated cervical spine stenosis, for which the patient had a medical history. The hypotension, which is atypical of acute methamphetamine toxicity, was thought to be due to fluid loss from the burns.

The patient's mental status gradually returned to normal by hospital day three. Despite still being quite ill, he insisted on being discharged and left the hospital against medical advice.

dial oxygen demand and at the same time causes coronary artery spasm and hypertension, leading to endothelial damage, endocarditis, and irreversible dilated cardiomyopathy (13). Noncardiogenic pulmonary edema and pulmonary hypertension can also occur in meth abusers.

There is a strong correlation and a complicated interplay between methamphetamine use and HIV, aside from the high-risk sexual behavior (14). Fatal cases in which patients ingest methamphetamine and HIV medications together indicate possible interaction between the two. The antiretroviral protease inhibitors ritonavir and delavirdine are substrates as well as inhibitors of CYP2D6 and may increase methamphetamine concentration in the body. HIV-related dementia can exacerbate dopamine neuron loss. Finally, studies of the feline immunodeficiency virus/cat model suggest methamphetamine increases the ability of the virus to replicate and mutate by 15-fold (15).

Methamphetamine smokers often lose their teeth abnormally fast, which is thought to be due to increased jaw clenching, poor personal hygiene, decreased saliva production, and deposition of crystal residue on the teeth (16). This is commonly known as "meth mouth."

### Laboratory detection

Many immunoassays are available for detecting amphetamine/methamphetamine and related sympathomimetic amines. Urine is generally the preferred sample due to a longer detection window. Because the immunoassays cannot differentiate methamphetamine from the other amines, a more specific test, usually gas chromatography/mass spectrometry (GC/MS), is required to confirm the presence of methamphetamine.

Recently, Woodworth et al. developed a method using the slope of the dose-response relationship to differentiate several reactive amines in the Emit II amphetamine/methamphetamine immunoassay (17). With optimal slope cutoffs determined by receiver-operating characteristic analysis, this method can enhance the detection specificity of amphetamine and methamphetamine vs. other sympathomimetic amines such as MDMA (ecstasy) and pseudoephedrine.

GC confirmation often involves extraction, cleavage of conjugates, and derivatization to increase the volatility of methamphetamine and amphetamine (18). Isolation can be achieved by either liquid-liquid extraction at alkaline pH or solid-phase extraction. Both enzymatic and acid hydrolysis methods have been used to cleave conjugates.

One potential pitfall of GC confirmation is

false-positive methamphetamine results in the presence of extremely high concentrations of other sympathomimetic amines. At an injection port temperature higher than 300 °C, amphetamine, ephedrine, or pseudoephedrine may be partly converted to methamphetamine after derivatization with heptafluorobutyric anhydride, 4-carbethoxyhexafluorobutryl chloride, or N-trifluoroacetyl-S-prolyl chloride (19–21). Therefore, the National Institute on Drug Abuse requires its certified laboratories to report a methamphetamine result as positive only when methamphetamine is greater than 500 ng/mL and when amphetamine is also present at higher than 200 ng/mL. Table 2 shows typical ions monitored for underivatized and derivatized methamphetamine.

In addition to GC/MS, other methods that have been used to confirm the presence of methamphetamine include GC with other detectors, high performance liquid chromatography (HPLC) with either ultraviolet or fluorescence detectors, high-performance thin-layer chromatography, capillary electrophoresis, and liquid chromatography/mass spectrometry (LC/MS) (18). In these procedures, methamphetamine and amphetamine may be detected with or without derivatization. Atmospheric pressure chemical ionization LC/MS was reported to produce very specific results for phenylisothiocyanate derivatives of methamphetamine, amphetamine, and other designer drugs simultaneously.

### Distinguishing legal and illegal use

Because clinical drugs such as Vicks Inhaler metabolize to the R-enantiomers of methamphetamine and amphetamine, it is sometimes necessary to discriminate between legitimate medication intake and illicit methamphetamine abuse. This is achieved by enantioselective GC or LC procedures. Enantiomers are separated either by a chiral stationary phase in chromatography or by an achiral stationary phase after derivatization with a chiral reagent. Mass spectrometry, a flame ionization detector, Fourier transform infrared spectroscopy, and an ultraviolet-diode array detector have been used for detection.

**Table 2. Typical Ions of Greatest Abundance of Underivatized and Derivatized Methamphetamine by EI-GC/MS (22)**

Analyte	Mass abundance ion
Underivatized	
methamphetamine	58, 91, 56, 65, 134, 51
Methamphetamine-AC	58, 100, 91, 65, 117, 191
Methamphetamine-HFB	254, 210, 118, 91, 169, 69
Methamphetamine-PFP	204, 160, 118, 91, 119, 205
Methamphetamine-TFA	154, 110, 118, 91, 69, 56
Methamphetamine-TMS	130, 73, 206, 91, 59, 114, 100

Because chiral procedures are not widely used in clinical and forensic laboratories, analytical differentiation of therapeutic use from illegal ingestion may not always be possible in a late phase of excretion. The parent drug, usually R-methamphetamine or its N-derivatives, may be metabolized quickly in the body. It is therefore important to obtain a detailed clinical history and medication list in these cases. Medically used methamphetamine derivatives include benzphetamine, famprofazone, fencamine, fenorex, and selegiline.

Specimens other than blood and urine have also been used to detect methamphetamine use. Among these, hair is unique in that it can provide a history of drug use for as long as several years after ingestion (23). Methamphetamine and amphetamine can be extracted from hair using various procedures, and then analyzed by immunoassay, GC, GC/MS, HPLC, LC/MS, and capillary electrophoresis. Because both parent compound and metabolites are absorbed into hair, it is possible to detect therapeutic parent drugs even after a long time, thus providing a means to discriminate between therapeutic and illicit use. At this time, however, hair analysis is not performed routinely in most clinical laboratories.

### Conclusion

To summarize, methamphetamine is a dangerous stimulant that can cause significant toxicity in many tissues. Laboratorians should have a thorough understanding of the capabilities and limitations of the analytical assays to help diagnose or identify methamphetamine overdose.

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## Adulterants

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specimen validity testing procedures specified by DHHS.

The collector should take steps prior to the collection to prevent and/or detect specimen adulteration. These steps include asking subjects to display the contents of their pockets to check for items that may be used to adulterate a specimen. In addition, subjects should leave outerwear (e.g., coats, jackets, sweaters) and bags (e.g., handbags, briefcases) outside the restroom used for the collection. The restroom should be prepared for the collection: Water sources should be disabled or secured; bluing agent should be added to the toilet bowl or tank; and items such as cleaning agents that could be used to adulterate the specimen should be removed. The collector should observe and note any unusual behavior that may indicate an attempt to tamper with the specimen.

Upon receiving the specimen, the collector should visually examine it for abnormal physical characteristics and measure the temperature of the urine within four minutes of collection. The temperature should be 90–100 °F.

### Specimen validity tests

DHHS guidelines require the laboratory to test all specimens for creatinine (with reflexed specific gravity), pH, and at least one oxidizing adulterant (such as nitrite, chromium, or halogens). Some laboratories use a colorimetric “general oxidant” test that is cross-reactive to many oxidizing compounds and usable on automated immunoassay instruments. The laboratory may also test for other adulterants.

The guidelines include criteria for laboratories to report specimens as substituted, dilute, invalid, or adulterated based on this validity testing.

If the creatinine concentration is less than 20 mg/dL, the specific gravity should be measured. A specimen is considered substituted if the creatinine is less than 2 mg/dL and specific gravity is either less than 1.0010 or greater than 1.0200. A specimen is considered dilute if the creatinine is less than 20 mg/dL and the specific gravity is less than 1.003, unless it meets the criteria for substitution (which is considered a more serious problem).

A specimen is reported as invalid if it exhibits discrepant creatinine and specific gravity results (for example, creatinine greater than 2 mg/dL and specific gravity less than 1.0010, or creatinine less than 2 mg/dL and specific gravity between 1.0010 and 1.0200).

It is also reported as invalid if pH is between 3 and 4.5 or between 9 and 11, and as adulterated if pH is less than 3 or greater than 11. A specimen is reported as adulterated if the laboratory identifies a specific adulterant by performing an initial test, followed by a confirmatory test using a different test method.

Household chemicals such as bleach, acid, soap, detergent, and vinegar can alter the pH of urine to a value outside the physiological range and can be detected by specimen validity tests. However, one study showed that small amounts of bleach had a negative effect on FPIA and RIA and on a cannabinoids gas chromatography/mass spectrometry (GC/MS) test (5). The amounts of bleach used in the study did not affect pH and were not detectable by odor. Specimens adulterated with liquid soap may exhibit unusual foaming when shaken. Visine eye drops cannot be detected by routine integrity testing.

Commercial urine adulterants such as Urine Luck, Klear, and Whizzies can cause false-negative results in drug tests. The presence of these compounds in urine may escape detection by routine

specimen integrity tests. Furthermore, although laboratories and researchers have identified the adulterants in such products and adapted their procedures to address them, adulterant product manufacturers have been known to alter the composition of the products to prevent detection.

### Adulteration with glutaraldehyde

Glutaraldehyde has been used as an adulterant to mask urine drug tests (6). One early commercial adulterant product containing glutaraldehyde was sold under the trade name of UrinAid. Glutaraldehyde solutions are also available in hospitals and clinics as cleaning or sterilizing agents or tissue fixatives. A 10% solution of glutaraldehyde is available from pharmacies as an over-the-counter medication for treatment of warts.

Glutaraldehyde at a concentration of 0.75% volume can lead to false-negative screening results for a cannabinoid test using the EMIT II drugs-of-abuse screen. George and Braithwaite found that EMIT II amphetamine, methadone, benzodiazepine, opiate, and cocaine metabolite tests were affected at a glutaraldehyde concentration between 1% and 2% (6). At a concentration of 2% by volume, the cocaine metabolite assay suffered an apparent 90% loss of sensitivity and the benzodiazepine assay had an 80% loss of sensitivity.

Glutaraldehyde also interfered with the CEDIA immunoassay for screening of abused drugs. Goldberger and Caplan reported that glutaraldehyde caused false-negative results with EMIT and false-positive PCP results with the FPIA (Abbott Laboratories) and KIMS (Kinetic Interaction of Microparticles in a Solution) immunoassay (Roche Diagnostics) (7).

### Tests for glutaraldehyde

Although the presence of glutaraldehyde can be detected by GC/MS, Wu et al. described a simple fluorometric method (8). When 0.5 mL of urine was heated with 1.0 mL of 7.7 mmol/L potassium dihydrogen phosphate (pH 3.0) saturated with diethylthio-barbituric acid for 1 hour at 96–98 °C on a heating block, a yellow-green fluorophore developed if glutaraldehyde was present. Shaking the specimen with n-butanol resulted in the transfer of this adduct to the organic layer, which can be viewed under long-wavelength ultraviolet light. Glutaraldehyde in urine can also be estimated using a fluorometer.

Because glutaraldehyde produces known abnormal immunoassay results for some drug classes, the DHHS guidelines allow labs to use these characteristic results as the initial test for glutaraldehyde. There are also commercial aldehyde tests for automated analyzers that may be used as the initial test.

GC/MS can be used as the confirmatory test needed to report a specimen as adulterated with glutaraldehyde.

### Adulteration with pyridinium chlorochromate

Wu et al. reported that the active ingredient of the commercial adulterant product, Urine Luck, was 200 mmol/L of pyridinium chlorochromate (PCC) (9). The authors reported that this adulterant caused a decrease in the response rate for all EMIT II drugs-of-abuse screens evaluated (amphetamine, cocaine, opiates, cannabinoids, and PCP) and for the morphine and cannabinoids assays of the Abuscreen (Abbott Laboratories). In contrast, this adulterant caused a higher response on the Abuscreen amphetamine assay and had no effect on the cocaine and PCP tests of either method. It did not alter GC/MS analysis of amphetamine, methamphetamine, benzoylecgonine, or PCP. However, it reduced the apparent concentrations of opiates and cannabinoids as determined by GC/MS.

### Tests for pyridinium chlorochromate

Wu et al. also described a protocol for detection of PCC using spot tests (9). The indicator solution contains 10 gm/L of 1,5-diphenylcarbazide in methanol. The indicator detects the presence of chromium ion and is colorless when prepared. Two drops of indicator solution are added to 1.0 mL of urine. If a reddish-purple color develops, the test is positive.

The addition of a few drops of PCC adulterated urine to approximately 0.5 mL of potassium iodide solution, followed by addition of a few drops of 2N hydrochloric acid, leads to liberation of iodine from potassium iodide, and this can also be used as a spot test to detect PCC (10). Four or five drops of 3% hydrogen peroxide added to 200  $\mu$ L of urine adulterated with PCC caused rapid formation of a dark brown color (due to reduction of heptavalent chromium by hydrogen peroxide); a dark brown precipitate appeared on standing. In contrast, unadulterated urine turned colorless after addition of hydrogen peroxide. There are commercial colorimetric reagents for chromium VI available for use on automated analyzers.

Confirmatory test methods include GC/MS for pyridine and high performance liquid chromatography (HPLC) or capillary electrophoresis (CE) for chromium VI. Other methods that can be used to identify chromium VI include inductively-coupled plasma mass spectrometry, multi-wavelength spectrometry (MWS), and ion chromatography (IC), but these techniques are not common in drug-testing laboratories.

## Adulteration with nitrite

The commercial adulterant Klear is sold as two microtubes containing 500 mg of white crystalline material that readily dissolves in urine without affecting color or temperature. Klear causes false-negative GC/MS results in confirmation testing for marijuana. ElSohly et al. first reported this product as potassium nitrite and provided evidence that nitrite leads to decomposition of ions of THC metabolites (THC-COOH) and its internal standard (11). (Whizzies is another commercial adulterant product that contains potassium nitrite.) The authors reported that using a bisulfite step at the beginning of sample preparation could eliminate such interference.

Tsai et al. further investigated the effect of nitrite on immunoassay screening of other drugs, including cocaine metabolites, morphine, THC-COOH, amphetamine, and phencyclidine (12). Nitrite at a concentration of 1.0 M had no effect on the Abuscreen assay. At a higher nitrite concentration, the amphetamine assay became more sensitive, and the THC metabolite assay became less sensitive. The GC/MS analyses of benzoylecgonine, morphine, amphetamine, methamphetamine, and phencyclidine were not affected, but recovery of THC-COOH was significantly reduced. Again, this interference could be eliminated by bisulfite treatment.

Urry et al. investigated the sources and resulting concentrations of nitrite in human urine, and determined that naturally occurring nitrite could be distinguished from adulteration (13). Nitrite may occur in urine in vivo because of pathological conditions such as sepsis, asthma, and rheumatoid arthritis; urinary tract infections; environmental sources such as air,

food, and water; and occupational exposure. In addition, there are medicines that metabolize to nitrite and medicines that stimulate the in vivo production of nitrite. Patients receiving medications such as nitroglycerine, isosorbide dinitrate, nitroprusside, and ranitidine may have increased nitrite levels in their blood.

The authors found that concentrations of nitrite were below 36 µg/mL in specimens cultured positive for microorganisms, and nitrite concentrations were below 6 µg/mL in patients receiving medications that are metabolized to nitrite. Nitrite concentrations were much higher in adulterated urine specimens (1910–12,200 µg/mL).

The DHHS guidelines specify a cutoff of 500 µg/mL for determining nitrite adulteration for both the initial and confirmatory tests. Because concentrations below 500 µg/mL have been shown to affect some drug tests, DHHS allows laboratories to report specimens as “invalid result” when nitrite is present at or above 200 µg/mL.

## Tests for nitrite

The addition of a few drops of nitrite-adulterated urine to 0.5 mL of 1% potassium permanganate solution, followed by addition of a few drops of 2N hydrochloric acid, turns a pink permanganate solution colorless with effervescence. The presence of very high glucose (greater than 1000 mg/dL) and ketone bodies may cause a false-positive result, but in this case it takes two to three minutes for the solution to turn colorless. On the other hand, if nitrite is present, the solution turns colorless immediately.

Another spot test involves addition of a few

**Table 1. Federal Cutoffs for Screening and Confirmation of Five Abused Drugs in Urine (3)**

Drug	Screening level	Confirmation level
Marijuana metabolites (Delta-9-tetrahydrocannabinol-9-carboxylic acid)	50 ng/mL	15 ng/mL
Cocaine metabolites (Benzoylecgonine)	300 ng/mL	150 ng/mL
Opiate metabolites (Morphine)	2,000 ng/mL <sup>1</sup>	
(Codeine)	2,000 ng/mL	
(6-Acetyl morphine) <sup>2</sup>	10 ng/mL	
Phencyclidine	25 ng/mL	25 ng/mL
Amphetamines (Amphetamine)	1,000 ng/mL	500 ng/mL
(Methamphetamine) <sup>3</sup>		500 ng/mL

<sup>1</sup> A combination of codeine and morphine may be detected by opiate immunoassays. These assays do not differentiate between codeine and morphine.

<sup>2</sup> Tested only when morphine concentration is greater than or equal to 2,000 ng/mL by the confirmatory test.

<sup>3</sup> Specimen must also contain amphetamine at a concentration greater than or equal to 200 ng/mL.

drops of nitrite-adulterated urine to 0.5 mL of 1% potassium iodide solution, followed by addition of a few drops of 2N hydrochloric acid. This results in immediate release of iodine from the colorless potassium iodide solution. Shaking this solution with *n*-butanol results in the transfer of iodine to the organic phase. If no nitrite is present, the potassium iodide solution remains colorless. There is no interference from high glucose or ketone bodies (10).

Nitrite can also be detected by diazotizing sulfanilamide and coupling the product with *N*-(1-naphthyl) ethylenediamine (i.e., the Griess reaction).

Commercial colorimetric reagents for the initial nitrite analysis are available for use on automated analyzers. There are also urinalysis dipsticks that test for nitrite (see below) that can be used as the initial test.

Test methods that can be used as to confirm nitrite include HPLC, CE, IC, and MWS. Singh et al. developed a quantitative HPLC assay for nitrite in urine using an IonPac AS 14 analytical column with an anion self-generating suppressor and conductivity detector (14). Using a single-point calibration, the assay was linear up to a nitrite concentration of 12,000 µg/mL. The detection limit was 30 µg/mL.

### Adulteration with peroxide/peroxidase

Stealth is a commercially available adulterant that consists of two vials, one containing a powder (peroxidase) and the other a liquid (hydrogen peroxide). Both products are added to the urine specimen. Cody et al. showed that this adulterant is capable of producing false-negative results in the Roche ONLINE and Microgenics CEDIA (cloned enzyme donor immunoassay) immunoassays when marijuana metabolites, LSD, and opiates are present at 125–150% of cutoff values (15).

Adulteration of an authentic positive specimen provided by a marijuana user caused that specimen to screen negative. Stealth effectively masked low concentrations of morphine (2500 ng/mL), but higher concentrations (6000 ng/mL) tested positive by both immunoassays. Cody et al. reported that GC/MS analysis of Stealth-adulterated urine using standard procedures for opiates proved unsuccessful in several cases, and in four of 12 cases neither the drug nor the internal standard was recovered (16).

### Tests for peroxide/peroxidase

Valtier and Cody described a rapid color test to detect Stealth in urine (17). Addition of 10 µL of urine to 50 µL of TMB (tetramethylbenzidine) working solution, followed by addition of 500 µL of 0.1 M phosphate buffer solution caused a dramatic color

change to dark brown. Peroxidase activity could also be monitored by using a spectrophotometer. Routine specimen validity checks (pH, creatinine, specific gravity, and temperature) did not detect the presence of Stealth.

Our experience shows that if a few drops of a urine specimen adulterated with Stealth are added to potassium dichromate, followed by a few drops of 2 N hydrochloric acid, a deep blue color develops immediately that usually fades with time. Peroxide/peroxidase may give a positive result using some general oxidant assays that are commercially available for use on automated analyzers. At this time, there is no confirmatory test for peroxide/peroxidase.

### Dipstick adulteration detection

Standard urinalysis test strips such as Multistix from Bayer Diagnostics and Chemstrips from Roche Diagnostics are sometimes used to detect adulterants in urine. However, among various pads in the test strip, only those for detection of nitrite, pH, and specific gravity have some value. The specific gravity test does not differentiate between specific gravity of 1.000 and 1.005, and therefore does not generally apply to substituted or diluted urine. The nitrite pad also detects a range that is clinically significant but well below the concentrations relevant for identifying invalid or adulterated specimens in workplace programs.

Recently, devices designed for on-site adulterant detection have become available. These dipsticks offer an advantage over spot tests because an adulteration check can also be performed at the collection site. Peace and Tarnai evaluated the performance of three on-site devices, Intect 7 (Branan Medical Corporation), MASK Ultrascreen (Kacey), and AdultaCheck 4 (Sciteck Diagnostics) (18). Intect 7 tests simultaneously for creatinine, nitrite, glutaraldehyde, pH, specific gravity, PCC, and bleach. Ultrascreen tests for creatinine, nitrite, pH, specific gravity, and oxidants. AdultaCheck 4 tests for creatinine, nitrite, glutaraldehyde, and pH.

The authors adulterated urine specimens with Stealth, Urine Luck, Instant Clean ADD-IT-ive (glutaraldehyde), and Klear at their optimum usage concentrations and concluded that Intect 7 was most sensitive and correctly identified these adulterants. AdultaCheck 4 did not detect Stealth, Urine Luck, or Instant Clean ADD-it-ive. Ultra Screen detected a broader range of adulterants than AdultaCheck 4; however, it detected these adulterants only at levels well above their optimum usage, making it less effective than Intect 7.

In contrast, King reported that AdultaCheck 4 is an excellent means of detecting contamination (19). AdultaCheck 4 and Intect 7 test strips can identify abnormally low creatinine or abnormal pH (20). These test strips can determine the range of creatinine or pH, but not the precise creatinine concentration or pH value.

### Legislative efforts

Even as researchers and drug-testing laboratories identify adulterants and adapt procedures to combat them, manufacturers alter the composition of adulteration products to avoid detection. Regulators and laboratories have expended a great deal of effort, money, and time to deal with the "moving target" approach of commercial adulterant manufacturers.

There has been some legislation on the state level concerning drug-test adulterants, although most states have not acted. It is illegal in states such as Texas to manufacture, sell, or distribute products to subvert drug tests. Other states have focused on the users of the products, making it a crime to attempt to defraud a drug test. Oregon denies workers' compensation to employees who attempt to subvert a test by adulterating a specimen.

At the federal level, a congressional hearing was held in May 2005 on the subversion of drug-testing programs (21). Federal agency officials, representatives from criminal justice systems, representatives from the drug-testing industry (such as drug-testing laboratories, a specimen collection organization, and program administrators), and others testified on the prevalence of commercial products for altering test results.

In March 2006, the National Drug Testing Integrity Act was introduced in Congress with the purpose of banning "any substance or device designed or intended to falsify, alter, or defraud a lawfully administered drug test for detecting a chemical or controlled substance in the body (22)."

### Conclusion

At this time, specimen validity testing is mandated only for testing of federal employees under DHHS guidelines. DOT, the federal agency with by far the largest number of federally regulated drug tests, does not require specimen validity testing. Many employers in the private sector have also chosen to not perform validity testing. Such tests may well be viewed as an unnecessary added expense in light of the small percentage of specimens reported as adulterated in workplace programs (23). However, while effective adulterants undermine testing programs by enabling drug users to alter their test results, even the misperception that an adulterant can

negate test results can decrease the deterrent effects of drug testing.

A focus on training and oversight of specimen collectors may lead to increased diligence in identifying and preventing adulteration at the collection site, while still protecting test subjects' privacy rights. Until legislation is enacted to prevent the ready availability of commercial adulterants, it is expected that the "cat and mouse" game between the drug-testing industry and adulterant product manufacturers will continue.

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