

Toxicology News

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Adulterants Continue to Challenge Laboratories

By Lou Jambor

Over the past few years, the sale of compounds touted to mask the presence of drugs in urine has increased considerably. A significant number of these urine additives have the ability to oxidize drug metabolites or inhibit immunoassay reactions. The purpose of this article is to identify these adulterants, describe their mode of action, and illustrate their prevalence.

The urine collection process in employee drug screening is generally well-controlled. However, few companies require direct observation during sample collection. Adulterants are packaged in small containers that can be smuggled into the collection facility without detection.

The adulteration of urine drug-testing specimens began rather crudely with the use of such common household items as bleach, vinegar, soap, ammonia, and the sodium hydroxide in such products as Drano. These adulterants achieved only limited success at masking detectable compounds. Adulteration has become more sophisticated with the introduction over the past several years of a wide variety of products designed for this purpose. Table 1 provides a partial listing of adulterants in the approximate chronological order of their appearance (1). These adulterants are readily available on the Internet or through such magazines as *High Times*. Their sale is an active, growing, and constantly changing business.

Surfactants

It has been known since the late 1980s that surfactants can inhibit the detection of marijuana metabolites, so it is not surprising that detergents and other surfactants were some of the first commercial adulterants. It has been postulated that the surfactants shield the marijuana metabolites from the anti-

Table 1. Commercial Adulterant Products (1)

Adulterant	Class	Active Ingredient
Mary Jane SuperClean 13	Surfactant	Lemon-scented detergent
UrinAid	Fixative	Glutaraldehyde
Clear Choice	Fixative	Glutaraldehyde/squalene
Urine Luck (pre-1998)	Acid	1.5 N HCl
Amber-13	Acid	1.7 N HCl
THC-Free	Acid	2.1 N HCl
Klear	Oxidant	Potassium nitrite
Whizzies	Oxidant	Sodium nitrite
Randy's Klear	Oxidant	Nitrite
Urine Luck (1998 and early 1999)	Oxidant	Pyridinium chlorochromate
Stealth* (recent version)	Oxidant	Peroxidase
LL-418	Oxidant	Pyridinium chlorochromate
Sweet Pea's Spoiler	Oxidant	Pyridinium chlorochromate
Randy Klear II	Oxidant	Pyridinium chlorochromate
Urine Luck* (recent version)	Oxidant	Pyridinium chlorochromate

* Both Urine Luck and Stealth were reformulated in autumn 2000. They are still under analysis to determine active ingredients and means of action.

bodies used in immunoassays. The lipophilic nature of the marijuana metabolites may cause these compounds to migrate inside of the micelles formed by the surfactant and shield it from the water-soluble antibodies (2).

It has been our experience that the surfactants can cause specimens with low levels of marijuana metabolites to screen negative by immunoassay. More commonly, we see the "soapy" specimen screen positive and confirm without problems by

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Difference Between Dig~~it~~oxin And Digoxin More Than “/t”

By Saeed A. Jortani and Roland Valdes Jr.

Digitalis drugs have been used medicinally for many centuries. Cardiac glycosides such as digitoxin and digoxin have been the major drugs indicated for the treatment of congestive heart failure. Significant advancements have been made in understanding the molecular mechanism of action of cardiac glycosides in the past 70 years.

Because of their narrow therapeutic indices, the serum concentrations of these drugs have been routinely monitored for at least the past 30 years. Intensive investigation and experience with immunoassays for digoxin and digitoxin have led to breakthroughs in the development of immunoassays in general as well as helped to develop the therapeutic drug monitoring practice. In this article, some aspects of pharmacokinetic, pharmacodynamic, and therapeutic monitoring of digitoxin and digoxin are compared and contrasted.

Digoxin and digitoxin are both composed of a lactone ring, a steroid nucleus, and three digitoxose sugars (1). Digoxin is structurally different from digitoxin by having an additional hydroxyl at position 12 in its steroid nucleus. After administration of a single dose of digitoxin, the main cardioactive metabolite in both serum and urine is digoxin, whereas at steady state, digoxin is a minor metabolite, constituting less than 1% of the administered dose (2).

Pharmacokinetics

In the United States, digoxin has been prescribed much more often than digitoxin for similar indications whereas in Europe, digitoxin is more popular. The two drugs are significantly different in their pharmacokinetic characteristics. For example, digitoxin's half-life is 100 to 200 hours and its time to steady state is approximately 1 month. Digoxin's half-life is shorter at 26 to 52 hours and its time to steady state is 1 week (3, 4).

Digoxin's volume of distribution is approximately 13 times greater than that of digitoxin, and its therapeutic range is about 10 times lower. Another main difference between these two is that 20% of digoxin and 97% of digitoxin are bound to plasma proteins (5).

Digoxin's urinary excretion accounts for 60% of the administered dose whereas digitoxin's fraction excreted by the kidneys is 32%. Both drugs are metabolized by sequential removal of their sugar moieties followed by extensive hydroxylation and conju-

gation to polar metabolites. Another significant pharmacokinetic difference between the two drugs is the fact that digitoxin is metabolized by the liver to a greater extent than digoxin.

Despite the fact that digoxin is relatively hydrophilic and digitoxin is relatively lipophilic, at the mid-therapeutic range, both drugs produce the same positive inotropic effect (as measured by systolic time intervals) (6). Changes in ejection fraction and circumferential fiber shortening (mVcf) are maximal at 4 to 6 hours after administration of either digoxin or digitoxin. These changes have been reported to return to baseline after 24 hours following digoxin administration but remain affected past 24 hours after digitoxin therapy (7).

Serum concentrations of digoxin have been shown to drop significantly after moderate physical activity (such as a 10-minute walk), while digitoxin's concentrations remain unchanged (8).

In a randomized, cross-over, single-blind study, Bussey *et al.* (9) reported that the therapeutic concentration of digitoxin was achieved in 14 out of 15 patients who demonstrated improved clinical response. In contrast, despite the fact that only 5 patients had serum digoxin concentrations in the therapeutic range, the CHF scores (an indication of pharmacodynamic efficacy) were the same as those patients treated with digitoxin. This indicates that the relationship between serum concentration and response is better-defined for digitoxin than for digoxin.

Toxicity ratings

A recently published retrospective study by Rover *et al.* (10) compared toxicity ratings by analyzing medical records of 147 hospitalized patients older than 60 years of age who were chronically managed with either of the two drugs. In this study, toxicity was defined as signs or symptoms such as anorexia, nausea, vomiting, diarrhea, cardiac dysrhythmias, and ECG changes. They showed that 7.6% of patients on digitoxin had a mention of these toxicity signs in their charts compared with 18.3% of those on digoxin. These results are consistent with other studies on patients on maintenance digitalis therapy, which have reported toxicity rates of 3.2% to 5.8% for digitoxin and 15.2% to 27.5% for digoxin.

Even though digoxin's only difference from digitoxin is an additional hydroxyl group—and the lack of “it” in its name—it exhibits a totally different pharmacokinetic profile. In patients with renal function insufficiencies, digitoxin is the drug of choice.

It appears that the old drug digitoxin is due for a “rediscovery” because it is as efficient as digoxin

pharmacodynamically and yet offers less toxicity and better serum concentration maintenance. Finally, it is worth mentioning that if digitoxin use rises in the United States, laboratorians should incorporate the necessary steps to ensure that when digitoxin is administered to a patient, the ordering and testing is for digitoxin. Ordering digoxin tests on patients on digitoxin and vice versa used to be a great problem in communities in which physicians ordered both drugs.

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Drug-Test Reporting Moves Beyond Positive and Negative

By Dave Armbruster

In the early days of forensic urine drug testing, for sake of argument let's say prior to 1988 and the creation of the Department of Health and Human Services (DHHS) National Laboratory Certification Program (NLCP), laboratories reported results as either simply "positive" or "negative." These definite, black-or-white results were supplied to clients who may have viewed them literally, often with little or no medical interpretation.

Fortunately for all concerned, reporting has become more sophisticated with time. Laboratories now have more options to better classify specimens and qualify analytical results and are encouraged to perform validity and adulteration testing. The analytical results are filtered through medical review officers (MROs) trained to determine if positive findings indicate drug abuse or have an acceptable medical explanation. Employers receiving the final reports have guidance for dealing with positive or suspect specimens.

Custody and control form

A new "Federal Drug Testing Custody and Control Form" (CCF) has been released. It has been available since August 1, 2000, and must be used with all specimens subject to federal drug-testing guidelines by July 31, 2001 (see www.health.org/workpl.htm for an example of the new form).

Two recent publications address the use of the new CCF and illustrate the increased level of sophistication of reporting forensic urine drug-test results: *Guidance for Using the "New" Federal Drug Testing Custody and Control Form* (DHHS Substance Abuse and Mental Health Services Administration, www.health.org/workplace/guidance.htm), and *Medical Review Officer Manual for Federal Workplace Drug Testing Programs* (DHHS, www.health.org/workplace/mromanual.htm).

This article briefly reviews the new reporting options and interpretations of test results. These instructions pertain only to "regulated" drug testing, that is, specimens subject to the federal NLCP testing guidelines. However, most unregulated drug-testing programs look to the federal program for guidance and tend to adopt the same standards.

As the CCF makes clear, there are actually two different types of drug-test reports. The laboratory produces the analytical result and reports it to the MRO. The MRO interprets the analytical result and

produces the final report given to clients.

Fatal flaws

The CCF is the most critical document in urine drug testing. Mistakes on the CCF can prevent a specimen from being tested. If the CCF contains "fatal flaws," the laboratory must report the specimen was "Rejected for Testing." Fatal, or nonrecoverable, flaws include: a mismatch between the ID number on the specimen bottle and that on the CCF; a broken or missing tamper-evident seal on the specimen bottle; the lack of a printed collector's name and signature; and insufficient specimen volume in the primary and split specimen bottles. The "Rejected for Testing" report means that the laboratory conducted no testing.

"Recoverable errors" on the CCF include: collector's signature missing but printed name present; specimen temperature box not marked; and subject social security or ID number not provided with no comment on the "Remarks" line. If the collection site sends an affidavit to the laboratory that provides the missing information or otherwise corrects the discrepancy, the CCF may be accepted and testing performed. If the CCF has a recoverable error, but the collector is unable to provide a memorandum to correct the discrepancy, a "Rejected for Testing" report is issued. The reason for rejection is stated on the "Remarks" line.

Negative vs. positive

If a specimen tests negative, the laboratory reports a "Negative" result. The MRO will also report a "Negative" result. However, a laboratory can also describe a negative result as "Dilute" if the creatinine test is <20 mg/dL and the specific gravity is <1.003 . The MRO makes a similar report.

A dilute specimen is still a valid negative, but the next time this subject is selected for a drug test, the employer may require that the specimen be collected under direct observation. A dilute specimen may indicate an exceptionally, but unintentionally, well-hydrated subject, or it could reflect a drug user who has purposely "water loaded" to dilute any drug or drug metabolite to a concentration below the testing cutoffs.

If a specimen screens and confirms positive, the laboratory issues a "Positive" result. The MRO receives the laboratory result and interviews the subject. If the subject provides a valid alternative medical explanation, the MRO reports a "Negative" result, thus reversing the laboratory's analytical report. The MRO's report does not invalidate the laboratory's analytical result, but interprets it in light of additional information.

If the subject does not have an acceptable explanation, the MRO reports a "Positive." A positive result may be reported for one or more drugs, specifically: marijuana metabolite, cocaine metabolite, PCP, codeine, morphine, 6-acetylmorphine, amphetamine, and methamphetamine. The requirement of reporting 6-acetylmorphine, the specific metabolite of heroin (diacetylmorphine), is also a fairly recent change.

Adulteration tests

Laboratories are now encouraged to perform a battery of adulteration tests, even following the traditional two-tiered approach of screening followed by confirmation, the same scheme used for the drug assays themselves. A laboratory may report a specimen as "Substituted" or "Diluted." A substituted report is given if the creatinine is ≤ 5 mg/dL and the specific gravity is ≤ 1.001 or ≥ 1.020 , and thus not consistent with a normal human urine. Adulterated applies if the nitrite concentration is ≥ 500 $\mu\text{g/mL}$, if the pH is ≤ 3 or ≥ 11 , or if a specific adulterant other than nitrite is detected and confirmed.

Even if the laboratory has obtained a confirmed positive result, the specimen is reported as substituted or adulterated, not as positive, as the substitution and adulteration test results take precedence over the drug-test result. The MRO reports substituted or adulterated specimens as "Refusal to Test," indicating that the subject has intentionally attempted to avoid detection. A laboratory may encounter a specimen that appears to be adulterated, for example, when drug is not found by GC/MS and the internal standard is reduced or not recovered. In this case, the laboratory may discuss the situation with the MRO and the specimen may be sent to a second laboratory for additional testing to identify an adulterant.

Invalid result

Laboratories have yet another option: "Invalid Result." An invalid result may be reported when: (1) the specimen is unsuitable for testing (physical appearance, such as the color or viscosity, is unacceptable), (2) a valid initial (immunoassay) result cannot be obtained, or (3) an unknown substance, such as an adulterant that cannot be identified, interferes with the GC/MS confirmatory test.

The MRO will interview the subject in these circumstances. The prescription drugs tolmetin, metronidazole, and ciprofloxacin can interfere with some immunoassays and could explain invalid initial test results. If an acceptable explanation for the invalid results reported by the laboratory cannot be

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Subscriber Questionnaire

To plan for 2001, the *CFT News* Editorial Advisory Board would like information on how the newsletter can best serve your needs. Please help us by answering the following questions.

Please mail or fax your questionnaire to Joanna Grimes, AACC, 2101 L Street, N.W., Suite 202, Washington, DC 20037; (202) 833-4568.

1. The purpose of *Clinical & Forensic Toxicology News* is to provide practical and timely information on the clinical, forensic, technical, and regulatory issues faced by toxicology laboratories. Does the newsletter achieve its purpose?

Circle one: Yes No

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Type of Laboratory: Medical Examiner's Office Crime Lab Hospital University
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Do you test for forensic purposes? Yes No

Do you perform confirmatory testing? Yes No

Thank you for your time and prompt response!

Test Reporting

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provided by the subject, the MRO reports "Test Canceled." In this case, the MRO directs the employer to collect a second specimen under direct observation. If the laboratory reports an invalid result for the second specimen, the MRO again reports "Test Canceled."

The MRO then recommends that the employer take no further action as it is assumed that the subject is taking a prescription drug that interferes with the testing or that an unknown endogenous substance prevents a valid drug analysis. The subject in these situations is not penalized, consistent with the philosophy of drug testing that subjects should be presumed to be drug-free in the absence of valid positive screening and confirmatory test results.

Retests

If the MRO feels that the original laboratory result may be scientifically inadequate, he or she may request a retest. The MRO may request that the original laboratory perform the retest or may request that an aliquot be sent to another NLCP-certified laboratory. An MRO-requested retest is performed on an aliquot from the primary bottle (Bottle A of a split collection). A subject, after being informed by an MRO that his or her test result is positive, may request that the secondary bottle (Bottle B of a split collection) be sent to another NLCP-certified laboratory for retesting.

In the case of an MRO-requested retest, the final report will be "Positive" if the laboratory reconfirms the original positive result. If the laboratory fails to reconfirm the original positive result, the MRO issues a final "Negative" report to the employer and contacts the appropriate regulatory office about the failure to reconfirm the positive result. The regulatory office will investigate the occurrence with the laboratory.

In the case of a subject-requested retest, the final report is "Reconfirmed" if a second laboratory reproduces the positive result. If the second laboratory is not able to reconfirm the original positive result, the MRO issues a final report of "Failed to Reconfirm." The appropriate regulatory office will investigate the "Failed to Reconfirm" finding with both laboratories. If the second laboratory believes that the drug or drug metabolite is present even though it cannot reconfirm the positive result, it may consult with the MRO and send the specimen to a third laboratory for retesting. Or, the MRO may direct the original laboratory to send an aliquot to a third laboratory for retesting.

More options

Clearly, laboratories are now expected to be more sophisticated and to be able to perform adulteration and validity testing on urine specimens. Suspect specimens may be rejected outright. Unusual specimens are reported as dilute or substituted. And, the detection and confirmation of a specific adulterant supersedes even the reporting of a positive drug result.

MROs also have more options when interpreting laboratory results. Laboratories and MROs are now more experienced and better able to report scientifically valid results on specimens that are physiologically valid and unadulterated as well as those that are suspect. There are still safeguards built into the federal drug-testing system to protect the subject. Subjects are given a second chance to provide a "normal" specimen. If abnormal results are obtained on a second specimen, but an adulterant or interferent cannot be specifically identified and confirmed, it is assumed that the subject's urine contains an unusual compound that precludes obtaining valid results, not that the subject is intentionally trying to avoid detection. Forensic urine drug testing is still not foolproof, but employers and subjects can rest assured that efforts are being made continually to ensure that it is scientifically valid, fair, and equitable.

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Limit of Absence Provides Assay Performance Measure

By Dave Armbruster

Good laboratory practice and regulatory standards, such as those required by CLIA '88 and CAP, call for evaluating the lower limit of assay performance. Traditionally, laboratorians have used the term "sensitivity" to refer to an assay's ability to measure very small concentrations of analyte and have equated it with the limit of detection (LOD). This terminology is probably inadvisable as "sensitivity" has at least three potential definitions: clinical sensitivity (the ability of an assay to correctly identify patients with a given disease by yielding a positive test result), analytical sensitivity (the change in analytical signal per change in analyte concentration), and LOD. The use of LOD seems preferable to "sensitivity" when defining the lowest concentration of analyte that an assay can reliably detect.

Common LOD determination

LOD has often been defined as the analyte concentration that can be distinguished from a blank or negative sample with 95% confidence. Most laboratories, however, do not determine the LOD in a fashion that is in keeping with the definition.

Often, the LOD is determined by analyzing blank or negative samples (often the zero calibrator) in replicate (usually $n = 20$). The mean and standard deviation (SD) of these assays are calculated. Typically, the LOD has then been calculated to be equal to the mean of the blank samples plus two SDs.

The number of replicates and SDs can be varied to attain a greater degree of statistical confidence. Three SDs instead of two captures a 99% confidence limit instead of only 95%. The use of an LOD calculated in this fashion is based on the presumption that an analytical signal greater than the LOD is not due to the background “noise” of the analytical system observed when no analyte is present, but instead is due to the presence of analyte.

Measuring nothing

It is helpful to know how much analytical signal an assay produces by testing a blank or negative sample. However, it's debatable whether this definition of LOD accurately predicts the lowest concentration of analyte that can be measured. Needleman and Romberg aptly observed that this statistical method “defines only the ability to measure *nothing*”(1).

Because no samples containing low concentrations of analyte are tested, this method of calculating the LOD leaves open the question of what concentration of analyte can be distinguished from the background analytical noise.

As analyte concentration is reduced, the corresponding measured assay signal will also decrease. Typically, at some point along a graph of analyte concentration versus signal, the linear relationship will be lost and signal response will become nonlinear. Unless an assay possesses exceptional analytical sensitivity, it will be unable to distinguish very dilute samples from one another. They will all appear to contain essentially equivalent analyte concentrations. As the analyte concentration approaches zero, the signal will become so weak that it will fall into the statistical distribution of signals produced by the inherent analytical noise of the system (as measured when a blank sample is tested). The lowest concentration of analyte that can be reliably distinguished from analytical noise cannot be determined without actually measuring the response to dilute analyte samples.

LOA

Recently, the International Union of Pure and Applied Chemistry (IUPAC) addressed the issue of characterizing lower limit of assay performance (2). IUPAC promotes the use of the “limit of absence” (LOA) along with the LOD, defining each of these parameters in a way that better reflects the performance of assays when blank and very dilute samples are tested. The LOA represents the amount of analytical signal that is measured when no analyte is present, that is, the analytical signal inherent in an assay system in the *absence* of analyte. The LOD is still defined as the lowest concentration of analyte that can be reliably distinguished from a blank or zero specimen. But the estimate of the IUPAC LOD is based on data from the analysis of specimens with very low concentrations of analyte, not solely from a statistical calculation from analyses of blank specimens.

Determining LOA

Using the IUPAC definition, the LOA is determined by measuring blank or negative samples, such as an assay's negative calibrator, in replicate (typically $n = 20$). The mean and SD of the analyses are calculated, and the LOA is calculated as equal to the mean value of analyte-free samples plus $1.645 SD_{LOA}$.

This formula is similar to the one used for the traditional LOD calculation except that instead of 2 SDs, 1.645 SDs is used. The 2 SD level represents a 95% confidence limit for a double-sided (two-tailed) distribution, while the 1.645 SD level defines the 95% confidence limit for a single-sided (one-tailed) distribution, as the distribution of values to the left (the “negative” side of the mean value) are not of interest. The major distinction between the LOA and the traditional definition of the LOD is that the LOA implies nothing about the minimum concentration of analyte that can be measured—it describes only the 95% limit for values that can result from analysis of a blank sample.

Under the IUPAC definition, the LOD is determined by measuring dilute solutions of the analyte, such as twofold or threefold dilutions of the first non-zero calibrator, in replicate (typically $n = 20$). The mean and SD of the analyses are calculated and the IUPAC LOD is calculated as: $LOD = LOA + 1.645 SD_{LOD}$.

As analyte concentration decreases and the limit of the assay's performance is approached, the SD (the variability of the measurement) increases until it stabilizes. That is, essentially the same SD is attained regardless of concentration. The SD that is

typical of these dilute solutions is chosen as SD_{LOD} , representing the SD at the limit of analytical performance.

Note that this is very similar to the formulas used for a traditional LOD calculation, and, as with the LOA, 1.645 SD is used to define the 95% confidence limit for a single-sided distribution. The significance is that the IUPAC LOD is based on *actual analysis* of dilute analyte solutions, not just on a statistical calculation based on the analysis of a blank or zero specimen. Thus, the LOA is essentially equivalent to the traditional LOD calculation, but, under the IUPAC approach, it serves as the basis of the LOD calculation.

The IUPAC LOD is guaranteed to be a higher value than the traditional LOD, and it is thus a more conservative, and realistic, estimate of the lowest analyte concentration that can be measured. The traditional LOD, based on measuring only a blank or zero sample, tends to yield an overly optimistic (that is, falsely low) estimate of the lowest measurable concentration.

Examples

As an example of an application of the IUPAC recommendation, the LOA and LOD were calculated as described above for four TDM enzyme immunoassays, carbamazepine, phenobarbital, phenytoin, and theophylline (3). Five dilute solutions of each drug were tested in replicate ($n = 20$) to establish the typical SD at low concentrations. The final values are given in Table 1.

Note that the LOAs above, representing values very similar to those derived from a traditional LOD determination, are consistently about half that of the IUPAC LODs. These LODs are 30- to 70-fold below the concentrations of the lower therapeutic ranges for these drugs.

The IUPAC LOD does not reflect assay accuracy at low drug concentrations. The accuracy of an assay is better addressed by the limit of quantitation (LOQ), which can require that the target concentration of a sample be recovered within some predetermined target range (e.g., 10% or 20% of true concentration) with a specified precision (%CV), although the LOQ may be equivalent to the LOD depending on how each parameter is defined.

For many drugs, the IUPAC LOA and LOD may be of little practical value simply because the

concentrations are so far below the clinically significant levels. However, the ability to clearly distinguish the presence from the absence of a drug may aid clinicians in ensuring that patients comply with a prescribed therapeutic regimen. For any drug assay, they are useful in defining the analytical performance at the lowest analyte concentrations.

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Adulterants

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gas chromatography/mass spectrometry (GC/MS). We have, however, encountered soapy specimens that produce very poor extractions.

Glutaraldehyde

Glutaraldehyde, marketed as UrinAid, was a popular adulterant in the early 1990s and is still occasionally seen today. It is effective in inhibiting the enzyme used in the Syva EMIT assays and rendering all compounds undetectable by EMIT. Specimens adulterated with glutaraldehyde exhibit EMIT ΔOD readings that are strongly negative, that is, the initial absorbance is much higher than the final absorbance. This unusual absorbance characteristic is the primary clue to the analyst that something is wrong with the specimen. Upon investigation, the characteristic odor of glutaraldehyde can often be detected. This odor has been described as that of overripe fruits and vegetables such as squash, pumpkins, or apples. Also, early versions of UrinAid produced pH levels that were slightly below normal.

Currently, many laboratories confirm the presence of glutaraldehyde by GC/MS, and its use appears to be declining. On rare occasions, specimens will screen positive for cannabinoids even after being adulterated with glutaraldehyde. GC/MS confirmation is not affected by the presence of glutaraldehyde.

Table 1. Examples of LOAs and LODs

Drug	LOA ($\mu\text{g/mL}$)	LOD ($\mu\text{g/mL}$)
Carbamazepine	0.0600	0.1201
Phenobarbital	0.0562	0.1401
Phenytoin	0.0723	0.1446
Theophylline	0.0725	0.1450

Acids

A series of adulterants using strong acids as the active ingredients have been marketed since the mid-1990s. Such products as Amber 13, THC Free, and an early version of Urine Luck were essentially hydrochloric acid at a concentration of 1–2 N.

Hydrochloric acid significantly interferes with the Syva EMIT assay for all analytes, often rendering the drugs undetectable. This effect is concentration-dependent as specimens will occasionally screen positive for an analyte and generally confirm by GC/MS without problems. Many laboratories currently test each specimen for abnormal pH. The U.S. Department of Health and Human Services and Department of Transportation have defined a specimen to be adulterated if the pH is less than or equal to 3 or greater than or equal to 11.

Nitrites

In late 1996, the adulteration of specimens with nitrites in products such as Klear became popular and presented toxicologists with an interesting challenge. Specimens adulterated with nitrites often screened positive for cannabinoids. The confirmation generated a result that appeared to be a poor extraction. Both carboxy-THC and the deuterated carboxy-THC (internal standard) were very poorly recovered. Samples with poor recovery are investigated in the laboratory, and it was quickly discovered that nitrites were present in the specimens. Most urinalysis testing strips contain a channel for nitrite analysis because nitrites may be elevated in urine specimens due to bacterial infections. The exceedingly high concentrations of nitrites (generally, greater than 1000 µg/mL) in these specimens readily gave strong positive readings.

By mid-1997, many laboratories had methods in place to accurately quantitate nitrites. Initial guidance from the Department of Transportation and the National Laboratory Certification Program was to use a cutoff of 1000 µg/mL for a positive nitrite. In September 1998, the cutoff was lowered to 500 µg/mL. Both of these levels are much higher than the levels that can be generated from bacterial infection, nitrite-containing foods, or medications, which generate levels of less than 50 µg/mL (3).

Nitrites are effective in eliminating carboxy-THC from urine; however, the effect of nitrite adulteration can be reversed in some cases through clean-up procedures (4, 5). It is rare for a nitrite-adulterated specimen to have a valid GC/MS confirmation without using a clean-up procedure. Nitrites also oxidize morphine but have no significant effect on codeine, amphetamines, phencyclidine, or benzoylecgonine.

Pyridinium chlorochromate

Pyridinium chlorochromate (PCC) was first seen as an adulterant in mid-1998. It was sold as the adulterant Urine Luck, which had previously been a strong acid. PCC is an oxidizing agent that readily converts secondary and tertiary alcohols to the corresponding ketone. Thus, PCC effectively oxidizes carboxy-THC. Specimens adulterated with PCC often screen positive for cannabinoids, but they generally do not confirm. In contrast to nitrite adulteration, the recovery of deuterated carboxy-THC is generally good.

This oxidizing agent reacts with the nitrite channel of urinalysis strips to give a purple color as opposed to the intense pink color seen in nitrite adulteration. (The MASK adulteration test strip distributed by the Lynn-Peavey company has separate test channels for nitrites, chromium(VI), glutaraldehyde, and pH.) The odor of pyridine can be detected in these specimens as the pyridinium ion converts to pyridine.

One version of this adulterant was formulated in approximately 0.1 M acid. This version often produced urine with abnormally low pH; however, it was replaced with a more neutral version. PCC appears only to affect the confirmation of carboxy-THC and has no significant effect on other analytes. Pyridine can readily be confirmed by GC/MS. By early 1999, many laboratories confirmed the presence of pyridine in specimens and reported them as adulterated.

Chromium(VI)

In mid-1999, the pyridinium ion was dropped from Urine Luck. The active ingredient is now chromium(VI), most likely potassium dichromate, which like pyridinium chlorochromate is a chromium(VI) species. Specimens adulterated with the new version of Urine Luck exhibit characteristics very similar to those caused by the previous version. Specimens screen positive for cannabinoids, and the confirmation result is generally negative with acceptable internal standard recovery. The nitrite channel of urinalysis strips shows a purple color that is even more intense than the color generated by earlier versions of Urine Luck. By late 1999, reagent manufacturers had developed kits for the detection of chromium(VI).

Prevalence

The prevalence of these adulterants in drug screening is significant. Studies performed by our laboratories from January 1999 through April 1999 determined that 0.54% of all specimens submitted for drug testing were adulterated. To put this number in perspective, this adulteration rate is greater than the

positivity rate for amphetamines. Nitrites were the most prevalent adulterant in 1999, detected in 0.34% of all specimens, followed by pyridinium chlorochromate (0.16%), glutaraldehyde (0.05%), and acids (0.01%).

In a study carried out from January 2000 to April 2000, the overall adulteration rate dropped significantly to 0.16% of all specimens. Nitrites were the leading adulterant at 0.07% of all specimens, followed by pyridinium chlorochromate (0.05%), chromium VI (estimated at 0.03%), acids (0.004%), and glutaraldehyde (0.001%).

A major reason for this drop in adulteration may be that the federal government has made a concerted effort to reduce the adulteration of specimens. The government has encouraged all certified laboratories to screen and confirm as many adulterants as possible. The consequence of an adulterated specimen is more severe to the employee than a positive test. The Department of Transportation treats an adulterated specimen as a refusal to test, which often results in employee termination. A positive test generally requires the individual to be evaluated by a substance abuse professional. It may also be possible that the reason for the decrease in adulteration is that there are new adulterants that are unknown to the laboratory or which cannot yet be detected.

Despite legislative initiatives pending in some states that would outlaw their sale, the proliferation of new and unique chemical mixtures used to adulterate urine drug-test specimens will no doubt con-

tinue. As creative entrepreneurs labor to develop products designed to thwart drug detection, drug-testing laboratories will need to be equally diligent and resourceful in their efforts to identify and overcome these attempts.

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