

# Toxicology News

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## EtG: Urinary Metabolite For Monitoring Alcohol Ingestion

By David J. Kuntz

**L**aboratory testing for alcohol has a long history. Early testing for blood alcohol was directed toward detecting impaired driving. Urine alcohol measurements started in the late 19th century, with breath alcohol testing beginning in the 1940s.

The measurement of ethanol in breath or blood suffers from a relatively short detection window. Blood has the additional disadvantage of requiring invasive sample collection by a trained phlebotomist. The interpretation of urine can be problematic because ethanol can be formed from the fermentation of glucose in some patients, such as in diabetics with microbially infected specimens (1). The value of a urine alcohol level is enhanced when there is objective evidence from law enforcement personnel who witnessed impaired driving or investigated an accident.

### Chronic ethanolism markers

Chronic ethanol abuse can be monitored through biological markers; these markers are widely used as a risk factor in the life insurance industry. Early indicators of liver damage include gamma glutamyltransferase, alanine aminotransferase, aspartate aminotransferase, and mean corpuscular volume. Protein structural changes are monitored with carbohydrate deficient transferrin, dolichols, and sialic acid. Direct markers of alcohol abuse include hemoglobin-associated acetaldehyde, the ratio of 5-hydroxyl-tryptophol to 5-hydroxyindole-3-acetic acid, and fatty acid ethyl esters. Each of these markers is present only in cases of high and chronic alcohol use, which limits their utility in an alcohol abstinence-monitoring program.

Alcohol abuse is a serious problem in the United States. A recent study of the American work-

force reported that 15% of employees were hung over, drank shortly before work, or were drinking or impaired while at work at least once in the prior year (2). Among chemically dependent health-care professionals in Alabama, alcohol was claimed to be the drug of choice 44% of the time (3). Because existing biochemical markers are relatively insensitive for detecting alcohol relapse and the window of detection for alcohol consumption is short, a more sensitive biological marker is required for monitoring alcohol abstinence.

Alcohol biomarkers can serve as objective measures and are helpful as outcome measures to evaluate new medications or behavioral interventions for alcohol problems, screens for individuals unwilling or unable to provide accurate self-reports of their drinking or its effects, and evidence of abstinence in individuals prohibited from drinking. They should be used in combination with a history or other confirmatory analytical techniques in order to distinguish inadvertent exposure to alcohol in various commercial products from conscious consumption of alcoholic beverages (4).

### A new marker

The newest biological marker for ethanol use is ethyl glucuronide (EtG). The proper nomenclature for EtG is ethyl  $\beta$ -D-6-glucosiduronic acid (molecular weight 222). EtG is a conjugated metabolite of ethanol, produced with activated glucuronic acid in the presence of membrane-bound mitochondrial uridine 5'-diphosphate glucuronyl transferase. This biochemical pathway represents approximately 0.02–0.06% of a dose of alcohol in hu-

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## Meconium Testing Provides Indication of Drug Exposure

By Loralie Langman

The first intestinal discharge from newborns is meconium, which is a viscous, dark green substance composed of intestinal secretions, desquamated squamous cells, lanugo hair, bile pigments, and blood. Meconium also contains pancreatic enzymes, free fatty acids, porphyrins, interleukin-8, and phospholipase A2 primary bile acids with a small quantity of secondary bile acids. Water is the major constituent, making up 85–95% of meconium (1).

The term meconium is derived from the Greek word “mekonion,” meaning poppy juice or opium. Aristotle is credited with noting the relationship between the presence of meconium in amniotic fluid and a sleepy fetal state in utero (1).

Illicit drug use during pregnancy is a major problem. Drug abuse during pregnancy is associated with significant perinatal complications, which include a high incidence of stillbirths, meconium-stained fluid, premature rupture of the membranes, maternal hemorrhage (placenta abruptio or placenta previa), and fetal distress (2). Drug-exposed neonates experience an increased mortality rate and increased morbidity, including asphyxia, prematurity, low birth weight, hyaline membrane disease, infections, aspiration pneumonia, cerebral infarction, abnormal heart rate and breathing patterns, and drug withdrawal (2).

### Drugs in meconium

The disposition of drugs in meconium is not well understood. The proposed mechanism is that the fetus excretes drugs into bile and amniotic fluid. Drugs accumulate in meconium either by direct deposition from bile or through swallowing of amniotic fluid (3, 4). The first evidence of meconium in the fetal intestine appears at the 10th to 12th week of gestation. It slowly moves into the colon by the 16th week (1). Therefore, the presence of drugs in meconium has been proposed to indicate in utero drug exposure up to five months before birth, a longer historical measure than is possible by urinalysis (3).

Unfortunately, identifying mothers who exposed their fetuses to drugs is not easy. Mothers often will not admit to the use of drugs because of denial about their addiction or fear of the consequences stemming from such admission. Many infants who were exposed to drugs in utero appear normal at birth and show no overt manifestations of drug effects. Thus, the most reliable means of identifying a drug-exposed infant may be drug testing, an objec-

tive means of determining drug exposure in both mother and infant. If hospital personnel suspect a mother has been using drugs, drug testing is necessary to document the infant's exposure.

### Analytical techniques

Meconium drug testing has been adapted to various analytical techniques, including radioimmunoassay, enzymeimmunoassay, fluorescence polarization immunoassay, high performance liquid chromatography, and gas chromatography. Drug confirmation by mass spectrometry is critical because some prescribed or semisynthetic drugs that the mother may have received during pregnancy can cross-react and cause a positive screening test. There are a variety of detection methods; their advantages and disadvantages have been described elsewhere (5, 6), and will not be discussed here.

### Sample collection

Meconium is collected directly from the diaper of the infant; thus, the collection is noninvasive.

About 1 g of meconium is needed for screening assays and 5 g for confirmation. As in any drug-testing specimen, the appropriate chain of custody for the collection, labeling, and transport should be strictly followed.

To prevent possible loss of drugs, meconium should be sent to the laboratory for processing as soon as it is collected. After standing at room temperature for 24 hours, meconium showed a decrease in cocaine and cannabinoid concentrations (2). Suspending meconium in an organic solvent, such as buffered methanol, may prevent decreases for as long as 72 hours (2). For prolonged storage, meconium should be frozen. Drugs are stable in meconium frozen at -15 °C for as long as nine months (2).

Meconium is easier to collect than neonatal urine, and the amount collected is usually sufficient for complete analysis, including confirmation.

Meconium testing does have some limitations. Meconium is usually passed by full-term newborns within 24 to 48 hours, after which the transition from a blackish-green color to a yellow color indicates the start of passing of neonatal stool. Infants with low birth weight (<1000 g) have been shown to pass their first meconium at a median age of three days. Thus, meconium collection can be missed because of delayed passage and may not be available soon after birth for early detection of intrauterine drug exposure.

Meconium is an unfamiliar matrix in the clinical laboratory, being a sticky material that is more difficult to work with than urine. All urine drugs-of-abuse screening assays used with meconium extracts must

be investigated for possible effects of the matrix on accuracy, precision, and assay linearity. Confirmation assays for meconium are more difficult than those for urine. Recoveries of drugs from meconium can be low (30–50%).

Many questions remain to be answered about the disposition of drugs in meconium. There is some debate as to which drug analytes are the most appropriate to measure in meconium, but Table 1 attempts to summarize the current knowledge.

### Interpretation

Overinterpretation of meconium data is a dan-

gerous practice. It is clear that there are matrix effects associated with the analysis of meconium, as with any biological fluid or tissue (7). Another important confounding factor is possible contamination of the specimen by urine. There are numerous reports of the specificity and sensitivity for different analytes using different testing methodologies (2, 8–11). The values for sensitivity and specificity differ depending on the prevalence, or pre-test probability.

There is a tremendous, and potentially inappropriate, value placed on a meconium result. Decisions about treatment or custody of the in-

## Meconium Test Data Provides Insight on Maternal Drug Use

By Jennifer A. Collins

Maternal substance abuse during pregnancy is a significant public health problem. Unfortunately, most estimates of drug use among pregnant women are based on national surveys that use self-reported information, which may be unreliable (1). Meconium testing has been shown to improve identification of prenatal drug exposure (2), which provides health-care professionals the opportunity for early intervention to mitigate its potential physical and developmental effects.

Although laboratory testing for drugs of abuse in meconium is more challenging than in other matrices, many hospital and reference laboratories offer testing services. The results from one reference laboratory are presented in Table 1 (3).

Although these positive rates give a reasonable estimate of individual drug prevalence, population prevalence is skewed by the fact that meconium screening is usually limited to mothers suspected of drug use, so the table includes results from an earlier year for an interesting comparison. During this eight-year span, the laboratory's meconium testing volume increased about 60%.

**Table 1. Meconium Percent Positives**

Drug	1998	2006
Overall combined	19.0	18.3
Amphetamines	7.3	19.2
Cocaine and metabolites	48.1	24.8
Opiates	13.2	11.1
Cannabinoids	30.1	46.2
Other*	1.3	7.2

\* Includes barbiturates, benzodiazepines, propoxyphene, methadone, and phencyclidine

Significant changes in the distribution of positives, especially with regard to amphetamines and cocaine, seem to parallel the increased incidence of methamphetamine use within the population as a whole. Also of note, the laboratory reported two confirmed positive phencyclidine results in 2006; there were no such positives in previous years. In both years, 7% to 10% of specimens were positive for multiple drugs.

This data differs significantly from the most recent National Survey on Drug Use and Health, in which 3.9% of pregnant women aged 15 to 44 years reported using illicit drugs in the previous month (4). Although the MEDTOX Laboratories' data represents results from selected high-risk populations, the difference implies that the actual magnitude of substance abuse within the maternal population may be higher than the national survey data found.

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**Table 1. Analytes to Measure in Meconium (4, 5, 7)**

Drug Class	Confirmation Compound
Cocaine	Cocaine Benzoylecgonine Cocaethylene <i>m</i> -Hydroxybenzoylecgonine
Opiates	Morphine Codeine 6-Monoacetylmorphine (6MAM) Hydromorphone Hydrocodone Oxycodone
Cannabinoids	9-Carboxy-11-nor-delta-9-THC 11-Hydroxy-delta-9-tetrahydrocannabinol 8,11-Dihydroxy-delta-9-tetrahydrocannabinol
Amphetamines	Amphetamine Methamphetamine MDMA (3,4-methylenedioxy-methamphetamine) MDA (3,4-methylenedioxyamphetamine) MDEA (3,4-methylenedioxy-N-ethylamphetamine)
Phencyclidine	PCP

fant, on occasion, have been based solely on the screening results. It is critical to remember that a positive test could indicate intrauterine drug exposure; however, a negative does not rule it out. It is clear that additional work is necessary to address these important issues and to improve our understanding of the disposition of drugs in meconium (7).

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## EtG Testing

*Continued from page 1*

mans (5). EtG was originally proposed as a detoxification pathway for alcohol in 1901 (6) and isolated in human urine in 1973 (7).

Interest in EtG has grown dramatically, changing it from a relatively unknown ethanol metabolite to an analyte of potentially major importance for monitoring alcohol exposure. Initial studies were performed in Europe in the 1990s, and interest spread into the United States in the past several years. In 2002, Swedish researchers reported a relatively simple analytical procedure based on the direct injection of urine diluted with a deuterated internal standard into an electrospray liquid chromatograph-mass spectrometer (8). The authors also reported that EtG was stable in urine for more than four days at room temperature and that EtG was not generated in alcohol-fortified urine samples.

### EtG stability and degradation

EtG has been shown experimentally to be stable in urine and resistant to heat, with EtG degradation observed in less than 1% of specimens. Because the degradation can be halted with the addition of chemical preservatives or by heating the urine specimens to 90 °C, specimen degradation is most likely due to the presence of bacteria or blood, which can introduce glucuronidase activity to degrade the glucuronide bond, resulting in lower quantitative levels with repeated analysis on consecutive days.

## Incidental exposure

Because EtG is an extremely sensitive measure for the detection of alcohol consumption, the incidental or unintentional consumption of alcohol is a significant concern for result interpretation. A small (and in some cases, large) amount of ethanol can be found as an ingredient in many foods, hygiene products (such as hair spray and hand sanitizers), over-the-counter products (such as cough syrup and mouthwash), and church wines for communion services. The use of these products can produce measurable levels of EtG in the urine.

When an individual is being monitored for abstinence, small amounts of alcohol can produce measurable levels of EtG and lead to possible consequences, such as the revocation of probation or loss of a professional or driver's license.

The following incidental exposure studies were performed with a limited number of subjects at Quest Diagnostics (9):

**Mouthwash:** Repeated use of mouthwash (containing 21.6% ethanol) over several days produced EtG levels up to 58 ng/mL.

**Cough and cold medicine:** Following three daily doses of one ounce of Vicks Nyquil (containing 25% alcohol), values of 246 ng/mL were obtained.

**"Non-alcoholic" beverages and communion wine:** Consumption of two 12-ounce "non-alcoholic" beers generated a 93-ng/mL value. Consumption of one teaspoonful of wine (9% ethanol) yielded maximum EtG levels of 77 ng/mL.

**Hand gel sanitizers:** Repeated application of a hand gel sanitizer (62% ethanol) throughout an eight-hour day resulted in a maximum concentration of 47 ng/mL.

## Active drinking and cutoff selection

Chronic use of alcohol has been reported to generate results for up to 80 hours (10). The detection periods for social drinking have not been well-defined. In-house studies with a limited number of subjects indicated that a single 12-ounce beer (3.2% ethanol) generated results greater than 100 ng/mL for approximately 18 hours, with a urinary peak value of nearly 4,000 ng/mL by four hours. A representative urinary profile is presented in Figure 1.

In a separate social drinking event, three six-ounce glasses of wine were consumed over a three-hour period (Figure 2). EtG levels were 229 ng/mL by the end of the first hour, with a maximum EtG level of 68,400 ng/mL by the fifth hour and a return to zero by approximately 40 hours.

Users of the EtG test can select a cutoff based on their requirements for monitoring alcohol abstinence. Programs that monitor employees with re-

sponsibilities for public safety generally select the most sensitive cutoff of 100 ng/mL. Program participants must know to avoid all products that contain alcohol to avoid possible low-level positive EtG results.

Commonly used alternative cutoffs are 250 and 500 ng/mL. These levels reduce the possibility of incidental alcohol causing positive results. An increase in the cutoff decreases the detection period of EtG, while diminishing the possibility of a positive test caused by incidental exposure to alcohol.

All positive results should be carefully evaluated by a medical review officer to determine if the positive result could be due to unknown ingestion of alcohol in an over-the-counter product, prescription drug, food, or other environmental exposure.

In an evaluation of 70,000 EtG screens, of those that were positive, 24.2% were detected between 100–250 ng/mL, 15.8% between 250–500 ng/mL, 12.6% between 500–1,000 ng/mL, and 47.7% greater than 1,000 ng/mL. These statistics translate into a 24.2% drop in the positive rate if the cutoff is raised

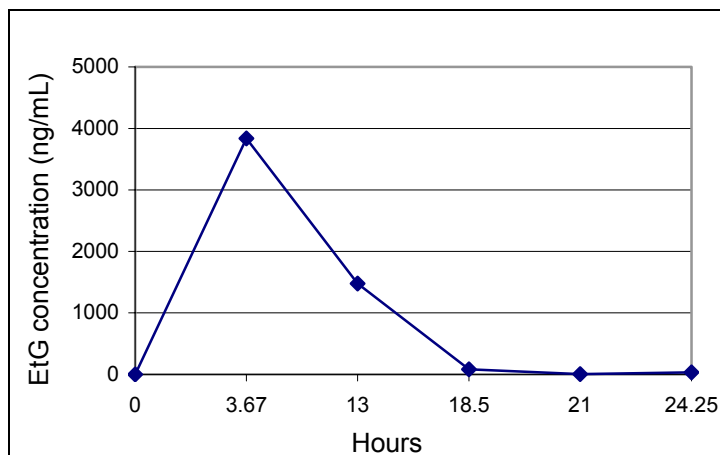


Figure 1. EtG Concentrations After One Beer

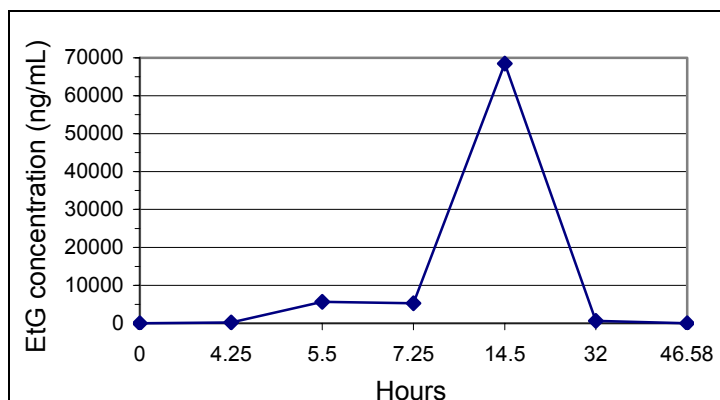


Figure 2. EtG Concentrations After Three Glasses of Wine over Three Hours

from 100 to 250 ng/mL and a 40% decrease if it is raised from 100 to 500 ng/mL (9).

### Conclusions

EtG is a sensitive indicator of alcohol exposure and metabolism and offers an extended window of detection for alcohol consumption that cannot be achieved by analyzing for ethanol in breath, blood, or urine. Because EtG is a sensitive marker of alcohol ingestion, careful evaluation of the results with the test subject and other corroborating analytical tests are necessary to exclude possible innocent or inadvertent alcohol ingestion. EtG offers the advantage of being a unique metabolic product that is not produced through fermentation processes that generate free alcohol. A positive EtG finding can confirm a urine ethanol positive or refute a positive urine ethanol result when fermentation conditions produce ethanol during storage of a urine sample.

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## SAMHSA Issues Advisory About Alcohol Biomarkers

By *Jeri D. Roper-Miller*

In September, the Substance Abuse and Mental Health Services Administration's Center for Substance Abuse Treatment published an advisory concerning alcohol biomarkers (1).

In addition to presenting current information on biomarkers that can indicate alcohol exposure or consumption, the advisory discusses the role of biomarkers in treatment and describes their advantages and limitations. It discusses well-known markers (liver enzymes and mean corpuscular volume), newer markers (carbohydrate-deficient transferrin and phosphatidyl ethanol), and controversial markers (ethyl glucuronide [EtG] and ethyl sulfate). When used in conjunction with patient interviews and screening tools, these measures can provide valuable information about a patient's alcohol use.

Concerning EtG, the advisory states: "Currently, the use of an EtG test in determining abstinence lacks sufficient proven specificity for use as primary or sole evidence that an individual prohibited from drinking, in a criminal justice or a regulatory compliance context, has truly been drinking. Legal or disciplinary action based solely on a positive EtG, or other test discussed in this advisory, is inappropriate and scientifically unsupportable at this time. These tests should currently be considered as potential valuable clinical tools, but their use in forensic settings is premature."

The complete advisory can be obtained at [www.kap.samhsa.gov/products/manuals/advisory/index.htm](http://www.kap.samhsa.gov/products/manuals/advisory/index.htm).

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## Quest Diagnostics Posts Workplace Drug-Testing Data

By David Armbruster

Drug testing of job applicants and employees is routine for many U.S. businesses and is mandated for many federal government agencies. Naturally, toxicologists wonder about the effectiveness of testing in detecting drug abuse. As a public service, Quest Diagnostics, the leading provider of employer drug-testing services, annually provides a statistical analysis, the Drug Testing Index, that recaps its previous year's experience with workplace testing.

The Drug Testing Index provides the positive test rates for the most common drugs of abuse and the proportion of positive results to the total number of drug tests performed. Results are divided into three major testing populations: (1) employees subject to testing by federal mandate, including those in public-safety positions (such as pilots, nuclear power plant employees, and bus and truck drivers), (2) employees in the general workforce subject to testing by employer policies, and (3) the combined workforce. The highlights of the index for 2005 are given here. Full details, including regional color graphic maps showing positive rates by drug type, are available online at [www.questdiagnostics.com](http://www.questdiagnostics.com).

The 2005 index reflects the results of more than 7.3 million workplace tests performed by Quest's national reference laboratory network. Last year's data indicates that workplace drug use was at the lowest level since the index was first published in 1988. The combined workforce had a 4.1% positive rate, compared with 4.5% in 2004 and 13.6% in 1988. Leading this encouraging trend was a significant decline in marijuana positives, which decreased by about 12% from 2004 to 2005. The annual total positive rate is summarized in Table 1 and the positive rates by worker category are given in Table 2.

### Amphetamine abuse

Amphetamine abuse, particularly methamphetamine abuse, is currently a major concern in the U.S., with articles on "crystal meth" appearing regularly in the media. The Quest data indicates that amphetamine drug-class positives decreased from 0.52% to 0.48% in the general workforce between 2004 and 2005, although the positive rate for amphetamines among workers in safety-sensitive positions subject to federally mandated drug testing rose 13%, from 0.31% to 0.35%, during that period. It is unclear whether the increase among federally regulated workers is significant or transient; however, a preliminary review of amphetamine positives by Quest

**Table 1. Annual Positive Rates for Combined Workforce**

1988	13.6%	1997	5.0%
1989	12.7%	1998	4.8%
1990	11.0%	1999	4.6%
1991	8.8%	2000	4.7%
1992	8.8%	2001	4.6%
1993	8.4%	2002	4.4%
1994	7.5%	2003	4.5%
1995	6.7%	2004	4.5%
1996	5.8%	2005	4.1%

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**Table 2. Positive Rates by Testing Category**

Testing Category	2005	2004	2003	2002	2001
Federally mandated workforce	2.3%	2.3%	2.5%	2.5%	2.9%
General workforce	4.5%	4.9%	5.0%	4.8%	4.9%
Combined workforce	4.1%	4.5%	4.5%	4.4%	4.6%

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for the first five months of 2006 measures the amphetamine positive rate at 0.28% for federally regulated workers and 0.43% in the general workforce.

Drilling down further into the amphetamines data indicates that methamphetamine use appears to be declining in both workforce categories. Dr. Barry Sample, director of science and technology for Quest Diagnostics' Employer Solutions Division said, "During 2005 we detected a downward trend in amphetamines positive test results in the general U.S. workforce and in 2006 the trend took hold among all U.S. workers. In the first five months of 2006, amphetamines drug-test positives declined to a three-and-a-half year low among both groups. This finding could reflect the increased efforts by federal, state, and local authorities to shut down clandestine methamphetamine laboratories."

The decline in positive rates extends across the other major drug categories monitored as well. Table 3 summarizes the positive rates for drug categories as well as specimen validity testing from 2001 through 2005. An additional interesting feature of the data is the positive rate mapping by ZIP code, which illustrates regional "hot spots" of drug use.

### Methaqualone testing

The Drug Testing Index indicates that routine testing continues for methaqualone. The positive rate for this drug has been 0.0% from 2001 through 2005. Methaqualone abuse is likely to remain a non-issue for the reasons stated in a 1998 article in this newsletter (1). Testing for methaqualone in workplace drug-screening programs appears to be a toxicological anachronism. Quest and other reference laboratories perform testing for this drug because clients request

**Table 3. Positive Rates by Drug Category**

(For general U.S. workforce as a percentage of all such tests. More than 6 million tests in 2005.)

Drug Category	2005	2004	2003	2002	2001
Amphetamines	0.48%	0.52%	0.49%	0.34%	0.29%
Barbiturates	0.25%	0.27%	0.29%	0.30%	0.34%
Benzodiazepines	0.58%	0.58%	0.60%	0.58%	0.60%
Cocaine	0.70%	0.72%	0.74%	0.71%	0.69%
Marijuana	2.54%	2.88%	2.96%	2.98%	3.17%
Methadone	0.23%	0.21%	0.20%	0.16%	0.13%
Opiates	0.32%	0.32%	0.34%	0.27%	0.29%
Phencyclidine	0.02%	0.01%	0.03%	0.02%	0.02%
Propoxyphene	0.57%	0.63%	0.67%	0.73%	0.52%
Invalid	0.16%	0.10%	0.10%	0.09%	N/A
Oxidizing adulterants (inc. nitrites)	0.00%	0.01%	0.02%	0.05%	0.05%
Substitution	0.01%	0.03%	0.03%	0.03%	0.03%

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it, and sometimes it is required by contracts between employers and unions or other organizations. Databases such as the Drug Testing Index can provide insight into prevalence and usage patterns that can highlight some practices in workplace drug testing that deserve scrutiny and reconsideration.

Toxicologists should keep in mind that the Drug Testing Index data is only from subjects whose specimens are tested by the Quest laboratory network; different populations may reflect demographic and geographic idiosyncrasies. However, because Quest is a high-volume test provider, it is likely that

this data does reflect trends in the U.S. workforce. Eternal vigilance to decrease drug abuse is still necessary as not all workers, and certainly not the general population, are subject to drug testing. Some drug-abusing individuals will always manage to avoid detection, ultimately to their own detriment and possibly with adverse consequences to others.

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