

# Toxicology News

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## Oral Fluid Recommended for Use as Drug-Testing Matrix

By Christine Moore

**I**n 2011, the Drug Testing Advisory Board of the Substance Abuse and Mental Health Services Administration recommended that oral fluid be allowed as a specimen in federal workplace drug-testing programs. The board had considered oral fluid samples previously, but turned them down because of disadvantages such as inadequate volume for analysis, drug absorption by collection pads, inability to create split samples, and concerns with marijuana analysis. The new recommendation is a recognition that technology has largely overcome these problems.

### Prevalence in drivers

The 2007 National Roadside Survey by the National Highway Traffic Safety Administration for the first time included the collection of biological specimens (1). Drivers were stopped randomly and, importantly, not “for cause.” In addition to a breath sample, the researchers took a blood sample and collected oral fluid with the Quantisal device (Immunoanalysis Corp., Pomona, Calif.). The samples were tested for more than 20 drug classes, comprising more than 75 drugs.

The results from the saliva samples indicated that 14.4% of night-time drivers were positive for drugs, compared with a rate of 13.8% from blood

samples, indicating that the oral fluid analysis was as good as or better than the blood analyses. The highest number of drug classes found in one saliva specimen was five: carisoprodol, hydrocodone, propoxyphene, tramadol, and nordiazepam. Some 98% of the drug positives from night-time drivers fell into five categories: marijuana (43%); cocaine (23%); pain medications, predominantly oxycodone and hydrocodone (18%); sedatives, predominantly benzodiazepines (7%); and amphetamines (7%).

### Advantages and drawbacks

Oral fluid has a major advantage over urine collection because the process can be observed easily, thereby minimizing opportunities for adulteration or substitution, which has become such a large issue in urinalysis. The collection can be carried out anywhere, is easy to administer, and is generally fast, although it can be slowed by “dry mouth” syndrome, which can be caused by various diseases, smoking, or opioid use. Other drawbacks that have traditionally plagued saliva testing, such as inadequate or unknown volume, drug recovery from a collection pad, and difficulty of analysis, have largely been overcome by technological developments in devices, assays, and laboratory instrumentation.

### Drug detection and concentrations

Oral fluid consists predominantly of water, with low concentrations of proteins, hormones, bacteria,

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## Dried Blood Spot Analysis Spreads to More Applications

By Ann-Sofie M.E. Ingels, Willy E. Lambert, and Christophe P. Stove

A dried blood spot (DBS) is capillary whole blood obtained by a finger or heel prick and collected on filter paper. DBS sampling has generally been used for newborn screening; however, it has recently received interest for use in therapeutic drug monitoring (TDM), clinical and preclinical studies, pharmacokinetics, and toxicology (1).

The DBS sampling technique ensures easy and rapid collection of a representative sample without stringent handling or storage requirements. These advantages over venipuncture make it a cost-effective choice for collection, transport, and storage of blood samples. Inherent to DBS sampling is the small volume used, ranging from 10 to 100  $\mu\text{L}$ , compared with 1 mL or more from venipuncture.

Although the small volume is advantageous when dealing with neonates and children, it can pose an analytical challenge, requiring efficient sample treatment and sensitive detection.

Tandem mass spectrometry coupled to liquid chromatography (LC-MS/MS) has been demonstrated to be highly suitable for the analysis of different pharmaceutical compounds or biomarkers in DBS, even at low concentration levels. The majority of DBS applications therefore use LC-MS/MS techniques, although LC coupled to ultraviolet detection (LC-UV) or fluorescence detection (LC-FLUO), and gas chromatography coupled to MS (GC-MS) or tandem MS (GC-MS/MS) have also been applied successfully. Automated techniques for the direct analysis of DBS are being developed, requiring no separation prior to detection (2–5).

### Derivatization

In general, DBS sample treatment starts with the extraction of the target analytes from the DBS with one or a mixture of organic solvents. In addition to extraction, the sample can also be derivatized before analysis to enhance volatility, to improve the chromatographic properties of the analytes of interest, and to increase detection sensitivity. Although derivatization is most widely used during sample preparation for GC-MS applications, LC-UV, LC-FLUO, or LC-MS/MS applications can also benefit from a derivatization step to improve the detection sensitivity by enhancing the UV properties, the fluorescence yield, or the mass spectrometric properties (for example, ionization efficiency,  $m/z$ ) of the target

analytes, respectively. In addition, direct MS/MS, without chromatographic separation, is a well-established technique for the quantitative determination of several biomarkers in DBS after derivatization. Most of these procedures are used to screen newborns for metabolic diseases (2–7).

### Direct derivatization

The derivatization procedure generally starts with extraction, followed by evaporation of the extraction solvent under a stream of nitrogen before addition of the derivatization reagent(s). Once the excess reagent has been removed, the redissolved or reconstituted derivatized extract is ready to be injected.

Because derivatization is laborious and tedious, LC and GC applications have been reported in which the reagents are applied to the extraction mixture without prior evaporation, followed by direct injection into the chromatographic system. Another convenient treatment procedure is direct derivatization, in which extraction solvents and derivatization reagents are applied simultaneously to the DBS. The extraction solvent needs to be selected based on its ability to allow the reaction to occur and result in acceptable extraction recovery of the derivatized target analyte (5). In “on spot” derivatization, derivatizing reagents are added without the use of any extraction solvent.

Direct derivatization has been used in several instances. For example, a method for the determination of the antidepressants fluoxetine, norfluoxetine, reboxetine, and paroxetine uses an aprotic organic solvent for extraction and direct derivatization, followed by a fast and sensitive GC-MS/MS technique (5).

An LC-MS/MS method for the determination of 3-hydroxyglutaric acid and glutaric acid involves direct derivatization of dried urine spots using an internal standard solution and derivatization reagents added successively, eliminating the extraction step (7). Recently, gamma-hydroxybutyric acid (GHB), a notorious club and date rape drug, was quantified using a sensitive GC-MS method following the application of a derivatization reagent mixture directly “on spot,” without an extraction solvent (3,4).

### Conclusion

In summary, DBS analysis has proven useful in a wide range of applications. Although LC-MS/MS is commonly used for DBS analysis, GC-MS/MS can also be used. To determine trace levels of low-molecular-weight compounds, a derivatization step may be necessary or may help to achieve adequate sensitivity.

The use of a single-step extraction and derivati-

zation or “on spot” derivatization can make sample preparation less time-consuming and laborious. This so-called direct derivatization may be considered for minimal, economical, and less time-consuming sample treatment. “On spot” derivatization, which was successfully applied to determination of GHB, is currently being investigated for the determination of other polar, low-molecular-weight compounds that pose extraction problems, such as  $\beta$ -hydroxybutyric acid; 1,4-butanediol; glycolic acid; and many others. These direct derivatization approaches could well increase in importance in the future, not only in GC, but also in LC-MS/MS and other MS-based approaches.

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## Ethylene Glycol Continues To Pose Threat of Poisoning

*By Donald L. Frederick*

Ethylene glycol poisoning continues to cause medical emergencies and claim lives worldwide despite the dissemination of information to the public about its toxicity. Although ingestion can have serious medical consequences, with early recognition the prognosis of treated patients is excellent. The most common source of ethylene glycol is commercial antifreeze solutions.

Ethylene glycol is a member of the group of glycols that includes propylene glycol, glycerol, diethylene glycol, and polyethylene glycol. Diethylene glycol is an example of a glycol ether that has significant toxicity and a history of mass poisonings.

An incident in 1937, in which more than 100 people died, propelled the passage of the Federal Food, Drug, and Cosmetic Act, which gave the U.S. Food and Drug Administration power over the marketing of drugs. The first report of a fatality caused by ethylene glycol was in 1930.

The American Association of Poison Control Centers listed 20 fatalities in 2010 from ethylene glycol as a single agent (1).

### Pharmacokinetics and metabolism

Ethylene glycol is rapidly absorbed from the gastrointestinal tract, reaching peak blood concentrations in one to four hours. It is highly water-soluble, with a volume of distribution of about 0.5–0.8 L/kg. Approximately 80% of the dose is metabolized by the liver and 20% is excreted unchanged.

Alcohol dehydrogenase is the hepatic enzyme responsible for the conversion of ethylene glycol to glycoaldehyde, which in turn is converted to glycolic acid (glycolate) via aldehyde dehydrogenase. The next step is elimination of the glycolate either by oxidation to glyoxylic acid and then to oxalate or by conversion to glycine and  $\alpha$ -hydroxy- $\beta$ -keto adipate, which are eliminated through the kidney.

The first stage of the elimination half-life of ethylene glycol is normally about three hours, but in some individuals can be up to 8.6 hours. This stage is the metabolism of ethylene glycol to glycolic acid. Reports of the half-life of glycolic acid vary widely, ranging from 2.3 to 18.5 hours (2).

Glycolic acid forms from ethylene glycol much faster than it is metabolized, so it accumulates, resulting in metabolic acidosis. The oxalate may form precipitates of calcium oxalate crystals, primarily in the renal tubules.

## Clinical presentation

As long ago as 1950, three stages of ethylene glycol toxicity were observed. Within the first several hours after ingestion, the initial phase is transient inebriation and euphoria similar to that caused by ethanol ingestion. Depending on the dose, ethylene glycol can irritate the gastrointestinal tract, leading to nausea and vomiting. As the metabolism proceeds, a metabolic acidosis develops that may be manifested by central nervous system (CNS) depression. This stage of the toxicity usually appears four to 12 hours after ingestion.

The second stage, at 12 to 24 hours after ingestion, consists of tachycardia with a severe metabolic acidosis that leads to hyperventilation and multiple-organ failure with secondary sequelae such as aspiration or congestive heart failure. Most deaths occur in this time period. After 24 hours, renal failure occurs with acute tubular necrosis. In severe poisonings, renal failure appears earlier and progresses to secondary sequelae.

In addition to the three well-known stages of toxicity, a delayed fourth stage of neurological sequelae has been reported that is thought to be from calcium oxalate damage to the cerebral vasculature (3). These authors describe case reports in which patients exhibit dysfunction of the lower cranial nerves that does not manifest until five to 20 days after ingestion. Calcium oxalate deposits have been found in the basal ganglia of these patients on autopsy. In one case, Parkinson's syndrome developed rapidly nine days after the patient presented with severe ethylene glycol poisoning.

## Treatment

Treatment regimens are tailored to the amount ingested and the time of ingestion. If the presentation is early, before the ethylene glycol has been metabolized to produce significant amounts of glycolic acid, treatment is based on inhibiting the metabolism by blocking the enzyme alcohol dehydrogenase.

Prior to the discovery of 4-methylpyrazole (fomepizole), ethanol was used in the treatment. Ethanol has a higher affinity for the metabolizing enzyme than ethylene glycol does, so a sufficient quantity of ethanol (maintaining the serum ethanol from 100–150 mg/dL) can block the metabolism of ethylene glycol to the toxic metabolites. The ethylene glycol can be slowly removed via renal elimination, with a half-life of 17 hours. More often, hemodialysis is used to remove the ethylene glycol, decreasing the half-life to less than three hours, while the ethanol blocks conversion to toxic metabolites. Hemodialysis has the advantage that it also removes glycolic acid and helps correct the acidosis.

If ethanol is used as an antidote, careful monitoring is required to maintain the proper blood ethanol level. The main side effect of this treatment is significant CNS depression.

## Fomepizole

Fomepizole has been available as an approved treatment for ethylene glycol toxicity since 1997. The American Academy of Clinical Toxicology issued practice guidelines for its use at that time (4). Serum concentrations of 8–25 mg/L have been described as effective, but fomepizole levels are rarely monitored because it lacks toxicity. The normal loading dose is 15 mg/kg, followed by 10 mg/kg every 12 hours for four doses. The dose is then increased to 15 mg/kg until the ethylene glycol levels fall below 20 mg/dL.

Fomepizole induces its own metabolism, hence the need for increasing the dose over time. Fomepizole is dialyzable, so it must be replaced if hemodialysis is used in the treatment, usually by increasing the frequency of doses from 12 hours to four hours.

Fomepizole increases the half-life of ethylene glycol from three hours to 17–20 hours. If the patient's renal function is impaired (creatinine > 1.5 mg/dL), the half-life may be as long as 29 to 49 hours, which prompts physicians to use hemodialysis to eliminate the unmetabolized ethylene glycol along with the toxic metabolites (5).

There is limited experience in the use of fomepizole in pediatric cases. A recent review examined 10 cases (6). One interesting finding is that ethylene glycol had a shorter half-life in pediatric patients than in adults who were receiving fomepizole treatment (9–15 hours for pediatric patients versus 20 hours in adult patients). This difference implies that pediatric patients can be treated with fomepizole alone without hemodialysis if their renal function is intact. A comparison of treatments found that fewer errors were made with the use of fomepizole than with ethanol. The errors that did occur were caused by excessive dose or delayed initiation (7).

## Later treatment

If treatment begins later after ingestion, and the patient presents with metabolic acidosis and an increased anion gap, the treatment first has to be directed toward the evaluation and correction of life-threatening conditions. The most serious are CNS depression, acute renal failure, and metabolic acidosis.

If ethylene glycol serum levels are available, they are helpful in determining the extent of ingestion. The glycolic acid levels are also helpful in determining the amount of toxic metabolites that have already formed. Because most institutions do not have these available, measurement of the pH helps

define the extent of the acidosis; however, as the time from ingestion increases, patients develop compensatory respiratory alkalosis. The anion gap may be helpful, but the pH changes will occur first. The difference between the calculated osmolal gap and the measured osmolal gap is another indication of the amount of ethylene glycol present in the blood when specific levels are not available. The conversion for ethylene glycol is only 8–10 mOsm per 50 mg/dL and therefore this approach is not as sensitive for ethylene glycol as it would be for other volatiles such as methanol.

### Laboratory analysis

No kit has been approved by the Food and Drug Administration (FDA) for ethylene glycol determination in human serum. An enzymatic kit based on glycerol dehydrogenase enzyme reagent from Catachem (Oxford, Conn.) is not FDA-approved but is available for laboratories to develop their own parameters for. A recent publication details how this kit can be optimized for use on an automated analyzer (8). The authors claim that their method almost entirely eliminates interferences from related compounds, and the instrument software prints error flags to identify which specimens need to be analyzed by a gas chromatography (GC) or gas chromatography-mass spectrometry (GC-MS) method.

Over the years, numerous GC and GC-MS methods for the analysis of ethylene glycol and related compounds have been described (9–11). Most of the GC-MS methods require simple protein precipitation followed by derivatization and selective ion monitoring on the MS. The most common interference is propylene glycol, which is often found in serum specimens due to its use as a preservative, emollient, and vehicle for oral and intravenous medications. Generally, propylene glycol is not very toxic because it is metabolized to lactate and pyruvate; however, case reports document fatalities from large ingestions (12). The GC and GC-MS methods rely on chromatographic separation of the two compounds.

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## Boston to Host SOFT Meeting

The annual meeting of the Society of Forensic Toxicologists will be held at the Boston Marriott Copley Place from June 30 to July 6.

For more information, visit the SOFT website: [www.soft-tox.org](http://www.soft-tox.org).

## CAP Offers New Proficiency Surveys and Added Analytes

The College of American Pathologists is offering four new proficiency testing surveys for toxicology and therapeutic drug monitoring (TDM) and is adding several new analytes to its existing surveys.

The Toxicology Resource Committee developed these surveys in response to evolving TDM and clinical and forensic toxicology practices. The committee used information from current subscribers to CAP surveys about their laboratory testing menus and practices to help direct the choice of analytes and kinds of challenges included in these surveys.

### Therapeutic Drug Monitoring, Extended (ZE)

Many laboratories have added clozapine, lamotrigine, levetiracetam, and oxcarbazepine metabolite to their testing menus. In response to the growing number of requests, this survey is specifically designed to address the proficiency testing needs of laboratories that are testing for these analytes. The survey offers two shipments per year consisting of three 5.0-mL serum specimens per shipment.

### Drug Monitoring for Pain Management (DMPM)

This survey is designed for laboratories that offer urine screening or confirmatory testing for monitoring compliance of patients receiving drugs for pain management. The survey includes analytes typically offered in pain management panels in concentrations and combinations designed to mimic what is commonly encountered in clinical practice.

It provides an educational benefit to laboratorians and pathologists who consult with pain management clinicians because it offers clinical cases and questions together with detailed follow-up information on how to interpret test results in the case study.

The targeted analytes are 6-acetylmorphine, amphetamines, barbiturates, benzodiazepines, buprenorphine and metabolite, carisoprodol (Meprobamate), cocaine and metabolites,  $\Delta$ -9-THC-COOH, fentanyl and metabolite, hydrocodone, hydromorphone, meperidine, methadone and metabolite, opiates (morphine and codeine), oxycodone, oxymorphone, propoxyphene and metabolite, and tramadol and metabolites.

The survey offers two shipments per year consisting of three 25-mL urine specimens per shipment.

### Trace Metals, Urine (TMU)

This survey is designed to address the proficiency testing needs of laboratories providing comprehensive analysis of trace metals in urine. The in-

cluded metals and the targeted concentrations challenge laboratories to detect the trace metals that may be present in urine following environmental or other exposures.

Targeted analytes include arsenic, chromium, copper, lead, mercury, selenium, thallium, and zinc. The survey offers two shipments per year consisting of three 10-mL urine challenges per shipment.

### Urine Drug Adulterant/Integrity Testing (DAI)

A major problem in forensic urine drug testing is the introduction of adulterants to alter or destroy drugs or drug metabolites that may be present in the specimen. This survey is designed for laboratories performing adulterant or integrity testing as part of their urine drug screening or confirmatory testing protocols.

Targeted challenges include pH, specific gravity, creatinine, nitrite, glutaraldehyde, and oxidants. The survey offers two shipments per year consisting of three 10-mL urine specimens per shipment.

### Analytes added to existing surveys

Cimetidine, methotrimeprazine, and norketamine have been added to the Toxicology (T) and Urine Toxicology (UT) surveys.

Fentanyl has been added to the CAP/AACC Urine Drug Testing, Screening (UDS) Survey and the CAP/AACC Urine Drug Testing, Screening, Limited (UDS6) Survey.

Please contact the College of American Pathologists at 800-323-4040 or 847-832-7000 for ordering information or if you have any questions about these surveys.

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## Oral Fluid

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food debris, and secretions from minor salivary glands. Drugs are generally detectable in oral fluid for one to two days after ingestion, but the length of time depends on the assay's limit of sensitivity and the drug involved. The National Institute on Drug Abuse has reported that the metabolite of marijuana (THC-COOH) can be detected in the oral fluid of chronic smokers for an average of 13 days (2).

Saliva is thought to reflect the free portion of drug circulating in the blood, provided that sufficient time has passed for drug equilibration. Specimens collected directly after use can be contaminated by drug remnants still in the mouth. Because the pH of saliva is lower than that of plasma, basic drugs are usually detected in higher concentrations in saliva than in blood.

Assuming that an oral fluid specimen is collected correctly, adequate volume is obtained, and sufficient time is allowed for any buffers to desorb drugs from the collection pad, then a routine testing laboratory now has the ability to carry out immunoassay screening for a wide range of drugs, as well as perform screening or confirmation procedures on gas chromatography-mass spectrometry (GC-MS) or liquid chromatography-MS/MS (LC-MS/MS) instruments. However, because the compounds detected in oral fluid are generally parent drugs and not metabolites, using immunoassay screens intended for urine screening causes problems. For example, cocaine appears as the parent drug to a far greater extent in oral fluid than in urine, so a screen that detects only the metabolite benzoylecgonine is an inadequate choice for oral fluid.

### Immunoassays

Immunoassays provide inexpensive initial screens that allow negative samples to be identified and discarded. Immunoassay screening is beneficial, operationally and financially, when sample volume reaches a level at which confirmatory test systems are overloaded by the number of tests or when the expected number of negative specimens is high. This screening may not be appropriate in clinical facilities where most specimens are positive for therapeutic drugs.

Many chemistry analyzers can be used for the analysis of oral fluid, as long as they have the capability of reading at 340 nm, adding two reagents, and maintaining a constant temperature. Several manufacturers have ready-to-use liquid reagent chemistries for oral fluid collected by various devices. All have advantages and disadvantages associated with the collection device itself, laboratory operations in the test facility, and immunoassay performance, but the range of oral fluid applications continues to increase.

Immunoassays for cocaine, methamphetamine, amphetamine, phencyclidine, marijuana, opiates, oxycodone, methadone, tramadol, buprenorphine, and carisoprodol are commercially available for oral fluid analysis in liquid reagent format. The availability of screening assays for chemistry analyzers will help urine laboratories move efficiently into oral fluid testing.

### Mass spectrometry

LC-MS/MS is being used increasingly in testing laboratories because it requires little or no sample preparation, requires no derivatization, and offers short run times. However, oral fluid samples may require special treatment. Collection device buffers often contain preservatives, anti-microbial agents, and

stabilizing compounds that generally make sample preparation necessary before direct injection. In addition, many drugs need to be extracted and concentrated prior to analysis, especially those that occur in very low concentration in oral fluid, such as benzodiazepines, carisoprodol, and morphine.

LC-MS/MS validation procedures should assess potential interference from structurally related drugs and metabolites, especially in the opioid class, which may produce the same mass spectral transitions. Various opioids can interfere with tandem mass spectral assays, and other problems can occur during routine analysis (3).

An additional concern in oral fluid is that the metabolites may differ from those in urine. The main metabolites of codeine, hydrocodone, and oxycodone are norcodeine, norhydrocodone, and noroxycodone, respectively. They are usually present in urine in concentrations higher than 10% of the parent compound. Of this group, only norcodeine is detected in oral fluid. Other traditional metabolites of these drugs are morphine, hydromorphone, and oxymorphone, respectively, which, if present in oral fluid as metabolites, are at concentrations less than 10% of the parent drug. The mass spectral transitions of these metabolites are likely to be similar in LC-MS/MS assays: The molecular weight of morphine, norcodeine, hydromorphone, and norhydrocodone is  $m/z$  285; the molecular weight of oxymorphone and noroxycodone is  $m/z$  301.

Although separations can be made based on retention time depending on chromatographic columns and flows, in routine work, analysts should be aware of the potential for misidentification. Therefore, extensive sample preparation is highly recommended, as is the incorporation of internal standards.

### Saliva compared with plasma

The theory behind using saliva as a sample stems from the belief that it is free (unbound) drug that is pharmacologically active, and over time the free fraction of a drug reaches equilibrium between the plasma and saliva. If the oral fluid level reflects the level circulating in the body, then it should be possible to develop therapeutic ranges based on oral fluid measurement after the drug has had adequate time to equilibrate. Saliva is not simply an ultra-filtrate of the plasma, and various factors affect the accumulation and diffusion of drugs in it. The saliva-to-plasma ratio depends on the physical and chemical properties of the drugs, the pH of the saliva, and contamination of the oral cavity if collection occurs immediately after drug intake. As a general rule, because saliva is more acidic than blood, basic drugs appear in higher concentrations in oral fluid.

In 2007, Langman published a comprehensive article on the use of oral fluid for therapeutic drug management in clinical and forensic toxicology (4). For chronic, frequent intake of a drug for which a therapeutic plasma range has already been established, the utility of saliva as an alternative sample matrix appears to have merit. The overall conclusion was that oral fluid has potential as an alternative matrix for therapeutic drug monitoring, and it currently may be applicable to some drugs but did not appear to be useful for most drugs that are therapeutically monitored.

The review found that drugs that are strongly protein bound, such as benzodiazepines, generally appear in lower concentrations in oral fluid. Pain medications, such as oxycodone, hydrocodone, and tramadol, appear in oral fluid at considerably higher concentrations than in blood. Carisoprodol is an exception, with a low saliva-to-plasma ratio.

### Specific drugs

**Marijuana:** A recent study collected oral fluid using the Quantisal device from 10 drug-free volunteers after they had been exposed to marijuana smoke

in locations in The Netherlands. All specimens were collected outside the store where the smoking was taking place. After only two hours of exposure, the active ingredient of marijuana, THC, was detected in some volunteers at levels above the new cut-offs that have been proposed by the Substance Abuse and Mental Health Services Administration for both screening and confirmatory assays. However, the metabolite THC-COOH was not detected in any of the specimens. The authors recommended that false-positive oral fluid results caused by passive exposure could be avoided by analyzing not only for THC, but also for the metabolite THC-COOH (5). Several publications describe procedures that can be used to enable detection of THC-COOH in oral fluid at picogram concentrations in routine analytical laboratories using two-dimensional GC-MS (2, 6), GC-MS/MS (7), and LC-MS/MS (8).

**Opiates:** Many studies have documented that the ingestion of poppy seeds by test subjects can cause positive morphine results in urine. To date, only one study has looked at whether poppy seed ingestion can cause positive results in oral fluid (9). It found that volunteers were positive for morphine in their oral fluid for up to one hour after eating poppy seeds at the screening concentration of 40 ng/mL. Recent publications have recommended that this cut-off be lowered to avoid missing true positives (10).

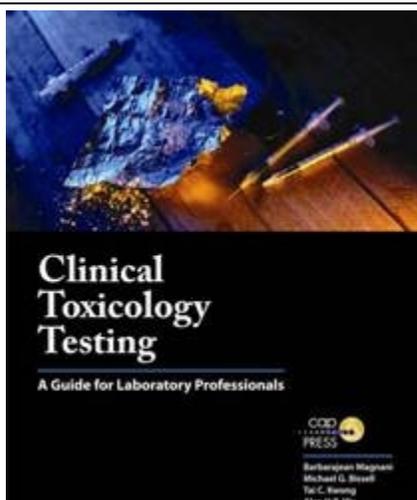
**Amphetamines:** It is recommended that tests include chiral enantiomers of both amphetamine and methamphetamine because *l*-methamphetamine appears to accumulate in oral fluid. This means that an immunoassay targeted at the *d*-form of the drug may not be positive, but one that cross-reacts with a significant percentage of the *l*-form is more likely to be.

### Summary

The utility of oral fluid is definitely increasing in many areas of drug testing. Technological advances in many areas, including collection, screening, confirmation, laboratory practice, metabolism, and interpretation, have added to the acceptance of saliva as an appropriate matrix. The major prescription medications for the treatment of pain appear to incorporate well into saliva, which could provide a new area for the introduction of oral fluid analysis.

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## New Clinical Toxicology Guide

*Clinical Toxicology Testing: A Guide for Laboratory Professionals* is a new book about the complex issues facing the laboratory director or pathologist whose facility offers toxicology services. Just published by the College of American Pathologists (CAP), this thorough reference book is designed to guide both experienced physicians and those in training through the pharmacological principles, testing menus, and methodologies of toxicology testing. The 300-page volume also covers workplace drug testing and relevant regulatory issues.

A table of contents and sample chapters are available at the CAP website.

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## Questions? Topics?

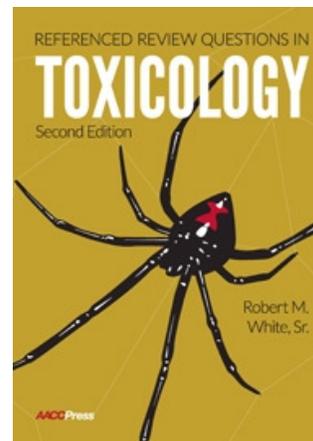
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### Learning objectives

After reading *Clinical & Forensic Toxicology News*, the reader should be able to:

- Describe emerging and changing trends in drug abuse, including new designer drugs, usage patterns, and contaminants/adulterants.
- Identify potential analytes (drugs, metabolites, biomarkers) of clinical and/or forensic significance.

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- Evaluate methodologies for their utility and limitations relative to the needs of toxicology labs.
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