Evaluation of a Human On-site Urine Multidrug Test for Emergency Use With Dogs

A rapid, human on-site urine multidrug test was used to screen canine urine samples for the presence of five illegal drugs and drugs from three commonly abused drug classes. Each sample was sent to a toxicology laboratory for gas chromatography/mass spectrometry (GC/MS) validation. On-site test results and GC/MS assays confirmed that the human on-site test kit did identify barbiturates, opiates, benzodiazepines, and amphetamines/methamphetamines in urine from dogs that had received these common illicit drugs/drug classes either intravenously and/or orally. However, neither the on-site test kit nor the GC/MS individual assays for marijuana or methadone, a synthetic opiate, were effective in identifying marijuana and methadone in urine from dogs with suspected or known exposure. No index of suspicion was seen for exposure to phencyclidines or cocaine during the study period, and no exposures were indicated by the on-site test results. Overall, the test is a rapid, readily available, affordable, and useful complement to the veterinarian’s clinical consideration and professional judgment. J Am Anim Hosp Assoc 2009;45:59-66.

Joan B. Teitler, DVM, MPVM

Introduction

Emergency room doctors, police, and drug rehabilitation programs routinely use rapid, human on-site urine multidrug tests for detection of illegal drugs. The test medium is easy to collect and can be analyzed within minutes of collection. On-site tests screen for commonly abused drug classes and individual drugs. Drug classes include barbiturates, benzodiazepines, and opiates. Individual drugs include amphetamine/methamphetamine, methadone (a synthetic opiate used in detoxification programs), cocaine, delta-nine-tetrahydrocannabinol (THC, the main psychoactive substance in marijuana), and phencyclidine.

Dogs are also exposed to these substances, and they exhibit some clinical signs similar to symptoms experienced by humans [Table 1]; however, interpreting clinical signs in dogs can be more challenging. In dogs, signs of intoxication may be subtle or contradictory and, therefore, confusing. Additionally, dog owners may be unaware of exposure or may be hesitant to volunteer an accurate history. To further complicate matters, a reliable on-site test has not been validated for use in dogs. Veterinarians in emergency situations frequently rely only on their clinical judgment for prognosis and planning treatments.

Gas chromatography/mass spectrometry (GC/MS) is the current method of drug detection for quantifying and identifying illegal drugs in body fluids from humans and other animals. Unfortunately, GC/MS is costly and time consuming. Urine samples have to be sent “off site” to a central laboratory, and GC/MS results may require several days to several weeks to be reported. Therefore, utilizing GC/MS in emergency use is impractical. Validating human rapid on-site urine drug tests for veterinary emergency use with dogs would allow immediate diagnosis and triage and facilitate emergency treat-
ment. Confirmation by GC/MS would only be necessary for medical/legal purposes.

The objectives of this study were to determine the efficacy of a human on-site urine multidrug test for screening dog urine for common illegal drugs and to validate the results with GC/MS.

Materials and Methods

Drug Test Kits

The on-site test used in this study screened for common drugs/drug classes including barbiturates, benzodiazepines, opiates, methadone (a synthetic opiate), amphetamine/methamphetamine, cocaine, THC, and phencyclidine. The test is an immunoassay that employs a chromatographic absorbent. Drugs or drug metabolites in a urine sample compete with a limited number of antibodies and conjugate dye-binding sites to selectively identify drugs of abuse [Figure 1].

Urine diffuses onto the porous membrane of the device by capillary action. Urine mixes with the antibody/dye conjugate and flows across the precoated membrane. When sample drug levels are below the detection concentrations,
Antibody/dye conjugate binds to the drug/protein conjugate immobilized in the binding sites in the “test region” (T); a colored test band is produced, which indicates a negative result. When sample drug concentrations are at or above the detection level, the free drug in the urine sample binds to the antibody/dye conjugate. This prevents the antibody/dye from binding to the drug/protein conjugate immobilized in the binding sites. This blocks the development of a distinct colored band, indicating a positive result. A single control colored band is always produced as a nonspecific reaction in the “control region” (C). A single control band confirms that the test is working and complete.21

On-site tests are qualitative and employ antibodies that may be shared by structurally related compounds. For example, a positive test for opiates occurs with all compounds in the same class. Gas chromatography/mass spectrometry is necessary to differentiate and quantitate the related compounds in the particular drug class.

Gas Chromatography/Mass Spectrometry Samples
To validate results, all specimens were mailed to the National Toxicology Laboratory (NTL) for screening with the GC/MS method.21 The United States Substance Abuse and Mental Health Services Administration (SAMHSA) specifies that GC/MS is the definitive “gold standard” for drug specimen identification and detection. All newly developed immunoassay tests are compared with GC/MS results to determine accuracy.22

Urine Samples
Urine samples were collected for testing between December 2003 and July 2006. Veterinarians from four urban veterinary emergency clinics collaborating with the study collected urine from 40 dogs. The urine was from three categories of dogs: those whose owners suspected drug ingestion; dogs that presented with clinical signs consistent with intoxication; and six control dogs [Figure 2].

Canine urine samples were also obtained from the Emergency/Intensive Care Service at the Veterinary Medical Teaching Hospital (VMTH), University of California at Davis. The Emergency Service at the VMTH obtained two samples during the study period from dogs that were presented for treatment by owners who suspected that their dogs had ingested THC. Forty-nine samples were collected from closed urinary collection systems from 27 dogs treated in the Intensive Care Unit (ICU). Because the urine was a waste by-product, neither owner consent nor the Institutional Animal Care and Use Committee approval was necessary.21 These subjects required medical treatment including intravenous and/or

---

**Figure 2**—Study configuration of 113 urine samples obtained from dogs tested with the on-site test.
oral administration of benzodiazepines, barbiturates, opiates, methadone, and/or multiple drug combinations of benzodiazepines, methadone, barbiturates, or other opiates. Urine was obtained by free catch from four volunteer dogs owned by emergency service technicians who provided informed consent to test for metabolites of dronabinol, a synthetic THC used to treat anorexia in human immunodeficiency virus and oncology patients. Dronabinol (2.5 mg) was administered to each dog orally as a legal substitute for THC to evaluate the on-site test’s THC binding site with a known dose. In humans, ingestion of 2.5 mg of dronabinol causes a positive result with the on-site THC binding site.

To rule out false positives or cross-reactions with random background drugs with the on-site test, 24 dogs that did not receive target drugs served as controls; six were from the urban emergency veterinary clinics, and an additional 18 samples were from the VMTH ICU. Except for the control dogs, all suspect and subject dogs tested were exhibiting clinical signs at the time of sample collection. The four volunteer dogs receiving dronabinol were mildly obtunded for 4 to 6 hours.

All samples were tested for target drugs with the on-site test within 6 hours of suspicion of drug administration, and they were mailed to the NTL for confirmation by GC/MS for each drug class within 24 hours. Two additional samples from dogs not participating in the study, but treated at the VMTH, were sent to the California Animal Health and Food Safety Laboratory System (CAHFS) for analysis by liquid chromatography/mass spectrometry (LC/MS) and an enzyme-linked immunosorbent assay (ELISA) test.

Statistics
Confidence intervals were calculated for test sensitivity and specificity [Table 2].

Results
Urban Emergency Veterinary Clinics
Of the 40 urine samples obtained from the urban emergency veterinary clinics, five were either of insufficient quantity or quality to process and were therefore excluded from the study. Twenty-five of the 40 urine samples were submitted from dogs suspected to have ingested THC; four urine samples were submitted from dogs suspected to have ingested amphetamines/methamphetamine; and six samples were submitted from control dogs [Table 2].

Test results from the 25 THC suspect samples showed either a strong red bar or a light pink bar in the THC binding site on the test probe, indicating a negative result for THC and/or THC metabolites in the urine. All 25 sample aliquots sent to the NTL were confirmed negative for THC by the GC/MS method.

The emergency veterinary clinics collected four urine samples from dogs suspected of ingesting amphetamines, and all four tested positive for amphetamines. On-site test results were confirmed positive for amphetamines by GC/MS.

Three control dogs were screened for THC and for cross-reacting substances, and results were negative with the on-site test and GC/MS. Three other control dogs tested negative for methamphetamines and amphetamines with the on-site kit and with GC/MS.

The VMTH Emergency Service
The VMTH Emergency Service collected 20 urine samples from dogs in the ICU that received benzodiazepines intravenously and/or orally. All samples were positive for benzodiazepines with the on-site test. These samples also tested positive with GC/MS for the benzodiazepine metabolites, oxazepam and temazepam.

Eleven VMTH dogs received barbiturates intravenously or orally. These samples were positive for barbiturates with the on-site test. All 11 samples tested positive for either phenobarbital or pentobarbital with GC/MS.

Fourteen dogs received opiates such as oxymorphone, hydromorphone, or morphine either intravenously or orally. The urine from these dogs tested positive with the on-site test for opiates. Gas chromatography/mass spectrometry confirmed the results more specifically as positive for morphine, oxymorphone, or hydromorphone. In contrast, the on-site test did not detect methadone in the urine of four dogs being treated in the VMTH ICU; these four dogs had received intravenous injections of methadone. Methadone was also not detected with GC/MS methadone assay at the standard GC/MS setting of 300 ng/mL [see Discussion, Table 2]. Urine from the four dogs that received the dronabinol orally was negative according to the on-site test. Results of GC/MS were also negative for THC. In addition, the two dogs included in the study that were suspected of ingesting THC also tested negative with the on-site test and the GC/MS THC assay.

One of the two dogs presenting to the VMTH for treatment outside the study parameters was witnessed by the owner to be ingesting hashish cookies, while the other owner expressed a suspicion that his dog had ingested THC. The urine sample from the dog that reportedly ingested hashish was submitted to CAHFS. The test result from CAHFS was negative on LC/MS THC assay at a 5-ng cutoff, but it was positive for THC on an ELISA test used at CAHFS. The other dog’s urine tested positive with the CAHFS LC/MS THC assay at a 5-ng minimal cutoff limit.

Eighteen urine samples were collected as controls from dogs in the VMTH ICU. Urine from four control dogs that had not received benzodiazepines tested negative for benzodiazepines with the on-site test, and urine tested negative with GC/MS assays for these drugs. Two urine samples from control dogs that did not receive barbiturates tested negative for barbiturates with the on-site test and the GC/MS barbiturate assay. The on-site kit and GC/MS tests were negative for opiates in eight urine samples from dogs that had not received opiates. Additionally, four dogs that had not received methadone tested negative for methadone with the on-site test and GC/MS methadone assay. No index of suspicion was seen for exposure to phencyclidines or...
cocaine during the study period, and no exposures were indicated by the on-site test results.

**Discussion**

The target drug/drug classes included on the on-site test represent a cross-section of the most commonly abused drugs on a national basis. Illegal drug use varies geographically, and this on-site test allows testing of a broad spectrum of illegal drugs with the most potential for drug ingestion by dogs. This on-site test was also selected for study because it is inexpensive and easy to use. Results were rapidly produced and easily interpreted. The on-site

---

**Table 2**

Test Results for On-site Urine Test Kits With Validation by Gas Chromatography/Mass Spectrometry (GC/MS)

<table>
<thead>
<tr>
<th>Drugs Identified by On-site Tests</th>
<th>GC/MS Confirmation Test Results</th>
<th>Proportion</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamines/Methamphetamines</td>
<td>4 positives</td>
<td>1.00</td>
<td>0.40-1.00</td>
</tr>
<tr>
<td>Barbiturates</td>
<td>11 positives</td>
<td>1.00</td>
<td>0.72-1.00</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>20 positives</td>
<td>1.00</td>
<td>0.83-1.00</td>
</tr>
<tr>
<td>Opiates</td>
<td>14 positives</td>
<td>1.00</td>
<td>0.77-1.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Drugs Not Identified by On-site Tests†</th>
<th>GC/MS Confirmation Test Results</th>
<th>Proportion</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>THC‡</td>
<td>27 negatives</td>
<td>1.00</td>
<td>0.00-0.13</td>
</tr>
<tr>
<td>Methadone</td>
<td>4 negatives§</td>
<td>1.00</td>
<td>0.00-0.60</td>
</tr>
<tr>
<td>Dronabinol</td>
<td>4 negatives</td>
<td>1.00</td>
<td>0.00-0.60</td>
</tr>
</tbody>
</table>

**Control Cases On-site Test Negative\** | Control Cases GC/MS Test Results | Proportion | 95% CI |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamines/Methamphetamines</td>
<td>3 negatives</td>
<td>1.00</td>
<td>0.29-1.00</td>
</tr>
<tr>
<td>Barbiturates</td>
<td>2 negatives</td>
<td>1.00</td>
<td>0.16-1.00</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>4 negatives</td>
<td>1.00</td>
<td>0.40-1.00</td>
</tr>
<tr>
<td>Methadone-synthetic</td>
<td>4 negatives</td>
<td>1.00</td>
<td>0.40-1.00</td>
</tr>
<tr>
<td>Opiates-from opium</td>
<td>8 negatives</td>
<td>1.00</td>
<td>0.63-1.00</td>
</tr>
<tr>
<td>THC</td>
<td>3 negatives</td>
<td>1.00</td>
<td>0.29-1.00</td>
</tr>
</tbody>
</table>

**No Exposures**

- Cocaine: None
- Phencyclidine: None

---

* CI=confidence interval
† Negative results imply either no exposure to target drug or no detection of drug in dog urine by the test kit.
‡ THC=delta-nine-tetrahydrocannabinol
§ See Discussion
\ Negative results indicate no cross reactivity with other drugs or substances.
test kit is also widely available over the counter at most national drug stores.

While the on-site test is easy to use, it carries a number of complicating factors. Accurate results depend on having a detectable amount of drug in the urine, an appropriate elapsed time from exposure to specimen collection [Table 3], and appropriate sample handling. For example, since THC adheres on glass and rubber stoppers, care must be taken to avoid using red-top tubes for specimen collection. In this study, each urine specimen was collected in a plastic vial while the dogs were exhibiting clinical signs and within 6 hours of suspicion of ingestion or after having been administered the drug.

Other complicating factors that influence target drug detection include species variation in drug biotransformation, specifically absorption, metabolism, and elimination of the target drugs. With regard to absorption, drugs that are lipid soluble tend to rapidly accumulate in body fat and may be released over days or weeks into the general circulation of the body. Other, more water-soluble drugs may be metabolized and eliminated within a few hours, making testing for urine metabolites more straightforward.

Drug metabolism also differs markedly between species. In dogs, early pharmacokinetic studies using intravenously injected carbon-14-labeled THC describe parallel production of several metabolites that are sequentially produced during liver passage. Forty percent to 45% of the carbon-14 dose in dogs was excreted in the urine, and 14% to 16.5% was excreted in the feces, and 55% was in bile within 5 days. At that time, 24% of the dose was unmetabolized and in the tissue. These data were consistent with enterohepatic recirculation of 10% to 15% of the metabolites. No THC or carbon-14-labeled drug was excreted unchanged. The THC in dog urine thus appears to be metabolized to a more complicated mixture of conjugated compounds—many of which are larger and more delicate than THC metabolites in human urine. This difference would explain why the on-site test, which is formulated to detect the human THC and THC metabolites in human urine, fails to detect the unique canine THC metabolites. Since 45% of the THC was excreted into the feces, fecal material might be a more appropriate test substrate.

Methadone is lipophilic, as is dronabinol and THC. Lipophils may be tissue bound to such an extent that too little illicit substance is eliminated through the urine for detection. Still another possibility, as with THC, is that the drugs may be metabolized and excreted in the urine in a form not uniformly recognized by the on-site test or GC/MS THC assay.

Individual differences in drug metabolism also might account for inconsistencies encountered with GC/MS THC testing. Even though GC/MS THC assay is the accepted test for canine exposure to THC, all of the GC/MS THC assay results included in the study were negative for THC and dronabinol, even though exposures were known. Study samples and results were variable when compared to

<table>
<thead>
<tr>
<th>Target Drugs</th>
<th>Approximate Times Target Drugs Can Be Detected in Human Urine</th>
<th>Minimum Detection Limit (Concentration) in Urine Used by On-site Test Kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamines/ Methamphetamine</td>
<td>From 0 to 2 d</td>
<td>1000 ng/mL</td>
</tr>
<tr>
<td>Barbiturates</td>
<td>From 0 to 21 d</td>
<td>200 ng/mL</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>From 0.5 to 7 d</td>
<td>200 ng/mL</td>
</tr>
<tr>
<td>Cocaine</td>
<td>From 0 to 3 d</td>
<td>300 ng/mL</td>
</tr>
<tr>
<td>Dronabinol</td>
<td>From 4 h to 5 wks</td>
<td>50 ng/mL</td>
</tr>
<tr>
<td>Methadone</td>
<td>From 0 to 8 d</td>
<td>300 ng/mL</td>
</tr>
<tr>
<td>Other opiates</td>
<td>From 0 to 2 d</td>
<td>300 ng/mL</td>
</tr>
<tr>
<td>Phencyclidines</td>
<td>From 0 to 8 d</td>
<td>25 ng/mL</td>
</tr>
<tr>
<td>THC*</td>
<td>From 4 h to 3 d</td>
<td>50 ng/mL</td>
</tr>
</tbody>
</table>

* THC=delta-nine-tetrahydrocannabinol
CAHFS results. The THC suspect that ingested hashish cookies tested negative on CAHFS LC/MS THC assay at a 5-ng detection limit, but the same dog tested positive on an ELISA test used at the CAHFS.4 Urine from the other dog admitted to the VMTH for THC ingestion, but not part of this study, was also submitted for testing by CAHFS. This urine sample tested positive with the LC/MS method for THC at a 5-ng minimal cutoff limit.

One of the methadone samples that tested negative with the standard GC/MS minimum detection limit of 300 ng/mL was reported to have “trace” amounts of methadone present. Based on these cases, some dogs do and some dogs do not excrete sufficient THC urine metabolites or methadone to be recognized by the GC/MS assay method used to identify these substances. The variable test results for THC and methadone imply that the gold standard GC/MS THC assay may not be a uniform test for these drugs in all dogs. All test results for THC and methadone should, therefore, be interpreted cautiously, with special consideration given to the individual dog’s clinical signs and history.

Surprisingly few confirmed exposures to the target drugs were reported. Given the prevalence of human drug abuse in our society and the shared close proximity of companion animals, particularly dogs, one might expect to encounter widespread drug intoxication among dogs.27 Indeed, the American Society for the Prevention of Cruelty to Animals (ASPCA) Poison Control Center reported 240 calls concerning canine THC ingestion between 1998 and 2002.14 Veterinarians participating in this study also expected to encounter many more cases of canine illicit drug ingestion. Since the current study results suggest that illicit drug exposure in dogs is relatively rare, these suspicions may be exaggerated. Having a validated on-site test available to veterinarians would eliminate this confusion.

Conclusion

The rapid on-site urine multidrug test effectively identified barbiturates, opiates, benzodiazepines, and amphetamines/methamphetamines in urine from dogs. Since these results were uniformly confirmed by GC/MS-specific assays, the on-site test for these target drugs is thus validated. The test is a rapid, readily available, affordable, and useful complement to the veterinarian’s clinical consideration and professional judgment. Further research, however, is necessary to develop accurate THC and methadone probes and assays.

Footnotes

a Barbiturates, benzodiazepines, and cannabis are commonly abused depressants, whereas amphetamines and methamphetamines are commonly abused stimulants. Opiates have been used for analgesia. Cocaine may have a biphasic effect with initial stimulation followed by depression. Phencyclidine is a dissociative anesthetic.6
b QuickScreen Pro Multi-Drug Screening Test; Phamatech, Inc., San Diego, CA 92121

c Hewlett Packard 5970 selective ion monitor. Standard operating procedures and protocols specified in current United States Substance Abuse and Mental Health Services Administration (SAMHSA) guidelines were followed. National Toxicology Laboratories, Bakersfield, CA 93304. (Also see reference 31.)
d Personal communication; Office of Research Animal Care and Use Committee, University of California, Davis, CA 95616

e Midazolam HCl injectable (25 mg/mL); Bedford Laboratories, Bedford, OH 44146 (Dose: 0.2 mg/kg IV)
Diazepam injectable (5 mg/mL); Hospira, Inc., Lake Forest, IL 60045 (Dose: 0.5 mg/kg IV)

f Phenobarbital injectable (130 mg/mL); Lumnial; Abbott Laboratories, Abbott Park, IL 60064 (Dose: 2.0 to 5.0 mg/kg IV)

Phenobarbital oral (16.2 mg, 32.4 mg, 64.8 mg); Vintage Pharmaceuticals, Huntsville, AL 35811 (Dose: 2.2 to 5.0 mg/kg)

Pentobarbital injectable: Nembutal sodium solution (50 mg/mL); Ovation Pharmaceuticals, Deerfield, IL 60015 (Dose: 20 mg/kg IV to effect)

g Oxymorphone: Opana (1 mg/mL); Endo Pharmaceuticals, Inc., Chadds Ford, PA 19317 (Dose: 0.05 mg/kg IV q 6 hours)

Hydromorphone HCl: injectable Dilaudid (4 mg/mL); Hospira, Lake Forest, IL 60045 (Dose: 0.05 mg/kg IV q 6 hours)

 Morphine sulfate oral: Oramorph (15 mg tablets); aaiPharma, Wilmington, NC 28405 (Dose: 1 to 3 mg/kg q 12 hours)

Morphone sulfate injectable (15 mg/mL); Baxter Healthcare Corp., Deerfield, IL 60015 (Dose: 0.1 to 0.25 mg/kg q 6 to 8 hours)

h Methadone HCl injectable (10 mg/mL); Xanodyne Pharmaceuticals, Inc., Newport, KY 41071-4563 (Dose: 0.5 mg/kg IV)

i Dronabinol oral: Marinol (2.5 mg tablets); Roxanne Laboratories, Columbus, OH 43216 (Dose: 2.5 to 5.0 mg/dog PO)

j Philip Kass, DVM, PhD, provided statistical analysis; Department of Population Health and Reproduction, University of California, Davis, CA 95616

k Products 105010 and 105015; California Animal Health and Safety Laboratory System, Davis, CA 95617

l Personal conversation; Office of Research Animal Care and Use Committee, University of California, Davis, CA 92121 www.phamatech.com

Acknowledgments

Multidrug test kits and GC/MS testing of samples were procured through the courtesy of Phamatech Corporation. Special thanks to Bruce Glasser and Maxine Davis, San Diego, CA 92121 www.phamatech.com.

Urine samples were collected by Contra Costa Veterinary Emergency Center; Solano-Napa Pet Emergency Clinic; Berkeley Pet Emergency Clinic; Pet Emergency and Specialty Center of San Rafael; and Sheryl Fullerton, Emergency Technician, and the ICU technicians at the VMTH. Special thanks to Dr. Janet Aldrich, Chief, Small Animal Emergency and Critical Care Service, for her support and encouragement.

References


27. Ibid 523-538.