Accuracy of Five On-Site Immunoassay Drugs-of-Abuse Testing Devices

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Abstract

Many current “on-site” urine drug-testing products claim performance equivalent to laboratory testing. Five commercially available products (PharmScreen™, Roche TestCup, Accusign™ DOA 2, Status DS™, and American Bio Medica-Rapid Drug Screen) were challenged with quality-control specimens of known drug metabolite concentrations, 25% above and 25% below the SAMHSA cutoffs, and with known positive and negative donor specimens previously analyzed by immunoassay and gas chromatography–mass spectrometry. The results indicate discrepancies between claims and performance for all products, particularly with amphetamines. The implications for employer-based drug testing are discussed.

Introduction

Non-instrumental “on-site” drugs-of-abuse testing devices (1–9) are widely marketed and gaining popularity in the criminal justice and law enforcement systems. In workplace testing, they promise virtually immediate results to employers eager to expedite the hiring decision. The Drug Testing Advisory Board (DTAB), a working advisory group to the Substance Abuse and Mental Health Services Administration (SAMHSA), has recently opened discussions for use of on-site devices in federally regulated workplace testing (10,11). Although on-site testing is not currently approved for federal workplaces, SAMHSA is considering the advantages and disadvantages of on-site devices and other “alternate technologies.”

There are important questions on how well the accuracy and reliability of on-site testing compare with the conventional laboratory testing process. The purpose of this study was to evaluate the accuracy of five commonly used (12–16) on-site devices by challenging performance above and below the SAMHSA cutoffs using quality-control (QC) specimens and comparing results of immunoassays and gas chromatography–mass spectrometry (GC–MS) performed in the laboratory with the new on-site technology using both positive and negative donor samples.

Materials and Methods

On-site testing devices

Five on-site drug testing products were selected for challenge. Selection was based on ease of availability, established history of commercial use, non-systematic observations on field characteristics, and cost. The stated cutoffs for all products for each of the five classes of drugs were the same as those required by SAMHSA standards (17). Four of the products included tests for the five NIDA classes of drugs: PharmScreen (PharmChem Laboratories, Menlo Park, CA), Roche TestCup (Roche Diagnostic Systems, Somerville, NJ), Status DS (Orion Diagnostics, Somerset, NJ), and American Bio Medica-Rapid Drug Screen (American Bio Medica Corp., Ancramdale, NY); one product tested for THC and cocaine only (Accusign DOA 2, Princeton BioMedi-tech, Princeton, NJ).

All products used membrane microparticle competitive enzyme immunoassay technology. In this technique, nitrocellulose strips are impregnated with a chemically labeled drug conjugate. If the drug or metabolite is present in the urine, it competes with drug conjugate for the antibody-binding sites. In the absence of drug in the urine test sample, the colored antibody migrates chromatographically to the immobilized drug conjugate zone to form a visible line as the antibody complexes with the drug conjugate. The formation of a visible line in the test region indicates a negative result. When native drug is present in the urine, it fills the limited antibody sites. This prevents attachment of the colored antibody conjugate to the antibody complexes with the drug conjugate. The formation of a visible line in the test region indicates a negative result. When native drug is present in the urine, it fills the limited antibody sites. This prevents attachment of the colored antibody conjugate in the test band region. Therefore, the absence of the colored band indicates a positive result.

1 National Institute on Drug Abuse, which preceded SAMHSA in responsibility for federal testing standards.
QC material

QC samples were purchased (Quality Assurance Corp., Augusta, GA). Each QC sample was prepared in pooled, filtered, human urine determined to be drug free by GC–MS. Sodium azide was added (0.1%, w/v) as a preservative. Specimens were spiked with drug or metabolite and prepared at 25% over and 25% below the current standard SAMHSA cutoffs by the manufacturer. Concentrations were verified by duplicate GC–MS analyses independently of the manufacturer (Table I). Methamphetamine was chosen as the target analyte in the amphetamines class because methamphetamine abuse is more prevalent in the workplace than is abuse of amphetamine. Most laboratory immunoassays' antibodies (e.g., EMIT®) are directed against methamphetamine.

Urine specimens

Human urine specimens consisted of pooled volunteer samples or donor samples submitted to Laboratory Specialists, Inc. (Gretna, LA). Samples were selected at random from those containing adequate volume (> 30 mL) for the required number of trials. Putative negative specimens (N = 20) were chosen from donor samples negative by immunoassay. Two additional negative specimens were chosen from volunteers' pooled urine shown to be drug free by GC–MS at the limit of detection (LOD) for each analyte. Immunoassay-positive aliquots were taken from aliquots submitted for GC–MS analysis, except for the Roche TestCup because of its large minimum 30-mL specimen volume. Aliquots for Roche TestCup analyses were retrieved from frozen storage (−20°C).

Testing

All testing was performed by one of the authors (EHT) to prevent variation in technique and to simulate “real life” use of the devices by a single user. Each test was performed according to the conditions and directions contained in the manufacturer’s package insert with respect to temperature, specimen volume, timing, and conditions for reading results. A positive result (presence of drug or metabolite at or above the cutoff) was determined by absence of a visible line. A visible line (even if faint) indicated a negative result.

Results

QC challenge

The results of the QC challenge at 25% above the stated cutoff are shown in Table II. No product gave a positive test result with methamphetamine present at 1374 ng/mL. This lack of sensitivity could be explained by the devices’ target analyte being amphetamine instead of methamphetamine. The package insert for Status DS (15) describes assays for both amphetamine and methamphetamine with cross-reactivity of 100% towards the target analytes amphetamine and methamphetamine at 1000 ng/mL. The PharmScreen package insert (12) states that a positive result does not occur up to 100 µg/mL (100,000 ng/mL) of methamphetamine. Roche (13) and American Bio Medica (16) did not provide data for the cross-reactivity of methamphetamine in their package inserts.

Table I. Quality-Control Challenge Concentrations*

<table>
<thead>
<tr>
<th>Drug/Metabolite</th>
<th>25% Below Cutoff† (ng/mL)</th>
<th>SAMHSA Cutoff‡ (ng/mL)</th>
<th>25% Above Cutoff§ (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methamphetamine</td>
<td>768</td>
<td>1000</td>
<td>1374</td>
</tr>
<tr>
<td>Benzoylecgonine</td>
<td>229</td>
<td>300</td>
<td>399</td>
</tr>
<tr>
<td>11-nor-9-carboxy THC†</td>
<td>36</td>
<td>50</td>
<td>63</td>
</tr>
<tr>
<td>Morphine</td>
<td>221</td>
<td>300</td>
<td>378</td>
</tr>
<tr>
<td>PCP</td>
<td>16</td>
<td>25</td>
<td>32</td>
</tr>
</tbody>
</table>

* Concentrations are the mean of two GC–MS analyses performed independently of the manufacturer.
† Lot number B035656.
‡ SAMHSA, Substance Abuse and Mental Health Services Administration. Federal regulations specify analysis of amphetamines and opiates as drug classes. Opine cutoff changed to 2000 ng/mL effective December 1, 1998.
§ Lot number B035657.
++ SAMHSA screening cutoff is based on many metabolites of THC, one of which is 11-nor-9-carboxy-THC.

Table II. Comparison of On-Site Devices at 25% Over the SAMHSA* Cutoffs†

<table>
<thead>
<tr>
<th>Methamphetamine</th>
<th>Benzoylecgonine</th>
<th>THCCOOH</th>
<th>Morphine</th>
<th>PCP</th>
</tr>
</thead>
<tbody>
<tr>
<td>PharmScreen</td>
<td>0 10</td>
<td>10 0</td>
<td>10 0</td>
<td>0</td>
</tr>
<tr>
<td>Roche Test Cup</td>
<td>0 10</td>
<td>10 0</td>
<td>10 0</td>
<td>0</td>
</tr>
<tr>
<td>Accusign DOA 2</td>
<td>N/A</td>
<td>10 0</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Status DS</td>
<td>0 10</td>
<td>10 0</td>
<td>10 0</td>
<td>4</td>
</tr>
<tr>
<td>American Bio Medica</td>
<td>0 10</td>
<td>10 0</td>
<td>10 0</td>
<td>4</td>
</tr>
</tbody>
</table>

* Substance Abuse and Mental Health Services Administration.
† N = 10 challenges per device.
‡ ± = Number of positive/negative results.
Using QC material spiked at 25% below the cutoff (Table III), all manufacturers showed methamphetamine at 768 ng/mL to be negative; all manufacturers showed benzoylecgonine at 229 ng/mL to be positive except one PharmScreen, which gave a negative result. Only the Roche TestCup correctly identified the THC metabolite present at 36 ng/mL as negative, whereas PharmScreen, Accusign DOA2, and Status DS all showed positives for THC metabolite present at 25% below the cutoff. American Bio Medica showed 5 of the 10 challenges as positive. Most samples for PCP below the cutoff were identified as negative except for one positive for Status DS and two positives for American Bio Medica.

Negative donor samples

The results of testing donor negative samples are shown in Table IV. Visible lines indicating negative test results, in addition to being contrary to what one would expect, were sometimes faint and/or hard to see. The American Bio Medica product produced lines for THC that were not as distinct or clear as other test lines (i.e., opiates, amphetamines, PCP, cocaine) for the same product. In addition, the THC test lines for American Bio Medica were not as clear or distinct as the THC lines for any of the other products. There were two false positives for THC with the American Bio Medica product; lines were not present for the two samples that were shown to be drug free by GC–MS at the LOD. In the remaining drug-free samples tested with the American Bio Medica device, THC and benzoylecgonine lines were faint.

Presumptive positive donor samples

There was much better correlation between laboratory tests and on-site device results among presumptive positive donor samples for THC metabolite, benzoylecgonine, and opiates (morphine) than for amphetamines, as shown in Table V. PCP showed some presumptive positives on the immunoassay performed in the laboratory that were later shown to be negative.

Table III. Comparison of On-Site Devices at 25% Below the SAMHSA* Cutoffs†

<table>
<thead>
<tr>
<th></th>
<th>Methamphetamine</th>
<th>Benzoylecgonine</th>
<th>THCCOOH</th>
<th>Morphine</th>
<th>PCP</th>
</tr>
</thead>
<tbody>
<tr>
<td>PharmScreen</td>
<td>10</td>
<td>0</td>
<td>9</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Roche Test Cup</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Accusign DOA 2</td>
<td>N/A</td>
<td>0</td>
<td>10</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Status DS</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>American Bio Medica</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>10</td>
</tr>
</tbody>
</table>

* Substance Abuse and Mental Health Services Administration.
† N = 10 challenges per device.
‡ -/+ = Number of negative/positive results.

Table IV. Comparison of Donor Samples (Negatives)*

<table>
<thead>
<tr>
<th></th>
<th>Amphetamines</th>
<th>Benzoylecgonine</th>
<th>THCCOOH</th>
<th>Opiates</th>
<th>PCP</th>
</tr>
</thead>
<tbody>
<tr>
<td>PharmScreen</td>
<td>22</td>
<td>0</td>
<td>22</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>Roche Test Cup</td>
<td>22</td>
<td>0</td>
<td>22</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>Accusign DOA 2</td>
<td>N/A</td>
<td>22</td>
<td>0</td>
<td>N/A</td>
<td>22</td>
</tr>
<tr>
<td>Status DS</td>
<td>22</td>
<td>0</td>
<td>22</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>American Bio Medica</td>
<td>22</td>
<td>0</td>
<td>22</td>
<td>0</td>
<td>22</td>
</tr>
</tbody>
</table>

* N = 22 challenges per device.
† -/+ = Number of negative/positive results.

Table V. Comparison of Immunoassay Positive Donor Samples for THC, Benzoylecgonine, Opiates, and PCP*†

<table>
<thead>
<tr>
<th></th>
<th>Benzoylecgonine</th>
<th>THCCOOH</th>
<th>Opiates*</th>
<th>PCP*</th>
</tr>
</thead>
<tbody>
<tr>
<td>PharmScreen</td>
<td>20</td>
<td>0</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>Roche Test Cup</td>
<td>20</td>
<td>0</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>Accusign DOA 2</td>
<td>20</td>
<td>0</td>
<td>20</td>
<td>N/A</td>
</tr>
<tr>
<td>Status DS</td>
<td>20</td>
<td>0</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>American Bio Medica</td>
<td>20</td>
<td>0</td>
<td>20</td>
<td>0</td>
</tr>
</tbody>
</table>

* N = 20 challenges per device, except PCP, which had 8.
† The negative results using Status DS and Roche Test Cup were negative for morphine and codeine by GC–MS.
‡ Six of the eight test samples for PCP were negative by GC–MS.
§ -/+ = Number of positive/negative results.
by GC-MS. Thus, the on-site devices showed good correlation with the GC-MS results for PCP.

Interferences
Amphetamines are commonly known to show the highest rate of immunoassay-positive, GC-MS-negative results in the laboratory. Table VI shows the comparison of the presumptive amphetamine-positive donor samples with the GC-MS data. Discrepancies between the two methods occurred in samples that contained other sympathomimetic amines or other cross-reactive compounds because the GC-MS was negative (none detected) on samples 2, 3, 4, 8, 9, 10, 11, 12, 14, 15, 16, 17, 18, and 20. As a brief exploration of test devices’ performances in this regard, we challenged the assays with various known cross-reactive substances, including pseudoephedrine, phenetermine, ephedrine, phenylpropanolamine, methylenedioxyamphetamine (MDMA), and methylenedioxyamphetamine (MDA).

The results of this challenge are shown in Table VII. No assay was affected by pseudoephedrine at 200,000 ng/mL or MDMA at 100,000 ng/mL. Phenetermine at 100,000 ng/mL produced false positives for all methods. It also produced a false-positive result at 10,000 ng/mL for PharmScreen, Status DS, and American Bio Medica, whereas the Roche TestCup was negative at this level. The American Bio Medica was resistant to ephedrine interference even at 1,000,000 ng/mL, whereas PharmScreen and Roche TestCup produced a positive result at 1,000,000 ng/mL but not at 100,000 ng/mL. Only the Roche TestCup had a problem with phenylpropanolamine, testing positive at 100,000 ng/mL and also at 10,000 ng/mL. The structural analogue to methamphetamine, MDMA, did not produce a positive result by any of the methods, but the structural analogue of amphetamine, MDA, showed a positive result at as low as 10,000 ng/mL for all assays.

Discussion
Employers use drug testing to reduce illicit drug use by employees with the expectation of reducing absenteeism and accident rates and improving productivity and safety. Current laboratory-based urine drug testing involves local specimen collection and careful chain-of-custody procedures for the sample during shipment to the laboratory and progress through testing; procedures have been refined during the past decade to minimize procedural and administrative errors. When each step of conventional drug testing is completed properly, the process and the test result are highly defensible. The price of quality is time delays: packaging and shipping a urine specimen to a distant site, accessioning the specimen into the laboratory information system, and the drug testing itself interpose hours or even days between the specimen collection and a “negative” test report to the employer. Confirmation of positives by GC-MS adds an additional day of testing.

In theory, on-site drug testing offers the advantages of speed and reduced cost over in-laboratory testing. However, employers and their agents, because of lack of time, resources, and/or scientific training, may be unable to test manufacturers'
A literature review did not yield information regarding per-}


target analyte was amphetamine. Nearly all QC samples con-


and 25% under the standard SAMHSA cutoffs. All assays missed}


assertions regarding sensitivity and specificity of the products.


A literature review did not yield information regarding perfor-}


tions of the initial result by labor-intensive, expensive GC–MS analysis at the laboratory.


One particular difficulty in all assays is the fact that the interpretation is subjective; the presence of a line, even a faint line, is inter-


In summary, on-site drug testing offers a rapid result; how-}


References


turer. Another issue concerns documentation of the test result.


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