Technical Note

Evaluation of the DrugCheck® 9 On-Site Immunoassay Test Cup According to a Standard Method Validation Protocol

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Abstract

Improvements have been made in recent years in on-site or point-of-care (POC) immunoassay testing devices. We evaluated the DrugCheck 9 cup, a new qualitative visually read, competitive binding, immunoassay cup that measures nine analytes. The study was performed according to the recent National Laboratory Certification Program (NLCP) guidelines for validating a laboratory-based immunoassay. The study included a linearity challenge with 5 replicates at concentrations 0, 25%, 50%, 75%, 100%, 125%, and 150% of the cutoff and also determination of the limit of detection. Interference (specificity) studies were included to evaluate the cross-reactivity of common structural analogues or others purported to interfere with the assay. The ability to differentiate positive and negative samples was evaluated by positive and negative quality control specimens and also parallel comparisons of laboratory verified positive and negative donor specimens previously analyzed by immunoassay and gas chromatography–mass spectrometry. The results indicated a high degree of correlation (97.6%) with the laboratory immunoassay and a high degree of specificity and sensitivity with extended linearity below the cutoff.

Introduction

Non-instrumental on-site or point-of-care (POC) drugs-of-abuse testing devices (1–14) are widely marketed in the criminal justice and law enforcement systems. In workplace testing, they promise virtually immediate results to employers eager to expedite the hiring decision. Improvements in technology in recent years have prompted the Drug Testing Advisory Board (DTAB), a working advisory group to the Substance Abuse and Mental Health Services Administration (SAMHSA), to review the use of on-site devices in federally regulated workplace testing (15,16). Although on-site testing is not currently approved for federal workplaces, SAMHSA is considering the advantages of on-site devices and other alternate technologies. Before the testing will be allowed, there will be the requirement to validate the method as is currently done with new laboratory-based immunoassay methods. The purpose of this study was to evaluate the accuracy and reliability of a widely used POC device, the DrugCheck 9 Cup (17), compared to the conventional laboratory testing process with a standard method validation protocol (18) by 1. challenging performance above and below the cutoff using quality control specimens; 2. comparing results of immunoassays and gas chromatography–mass spectrometry (GC–MS) performed in the laboratory with the POC device, using both positive and negative donor samples; and 3. evaluating the interference and cross-reactivity with common structural analogues.

Materials and Methods

On-site testing device

The DrugCheck 9 Cup (Lot # 35631) was obtained from Drug Free Enterprises (Agoura Hills, CA). The stated cutoff for amphetamine, methamphetamine, carboxy-tetrahydrocannabinol (THC), cocaine metabolite (benzoylecgonine), and PCP were the same as those required by SAMHSA standards (18). The cutoff for opiates (morphine), benzodiazepines (oxazepam), and barbiturates (secobarbital) was 300 ng/mL while the cutoff for the tricyclic antidepressants (amitriptyline/nortriptyline) was 1000 ng/mL.

The cup used membrane microparticle competitive enzyme immunoassay technology. In this technique, nitrocellulose strips were impregnated with a chemically labeled drug conjugate. If the drug/metabolite is present in the urine, it competed with drug conjugate for the antibody binding sites. In the

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absence of drug in the urine test sample, the colored anti-drug antibody migrated chromatographically to the immobilized drug conjugate zone to form a visible rose-pink line as the antibody complexes with the drug conjugate. The formation of a visible line in the test region indicated a negative result. When native drug is present in the urine, it filled the limited antibody sites. This prevented attachment of the colored antibody conjugate in the test band region. Therefore, the absence of the colored band indicated a positive result.

GC–MS methods

Prior to extraction, opiates and benzodiazepines were hydrolyzed with β-glucuronidase and THC acid was hydrolyzed with 10N KOH. The amphetamine, methamphetamine, THC acid, benzoylecgonine, barbiturates, and benzodiazepines specimens were extracted from urine using solid-phase extraction with Detectabuse (Biochemical Diagnostics, Edgewood, NY) extraction columns. Opiates were extracted with CleanScreen (United Chemical Technologies, Bristol, PA) columns. Amphetamine and methamphetamine were derivatized with 4-carbethoxyhexafluorobutyryl chloride (4-CB, United Chemical Technologies); THC acid, benzoylecgonine, and benzodiazepines were derivatized with MTBSTFA (Pierce Chemical, Rockford, IL); and opiates were derivatized with BSTFA (Pierce Chemical). A single-point calibrator at the cutoff was used for all assays. PCP was extracted using a liquid–liquid extraction with chlorobutane. We used the 5971 and 5973 GC–MS (Agilent, Palo Alto, CA) with RTX5-MS fused-silica columns (Restek, State College, PA) and analyzed under selected ion monitoring (SIM). Quantitation was determined by using a deuterated internal standard for each assay and comparing the peak-area ratio of the unknown to that of the calibrator at the cutoff. The limits of detection (LOD)/quantitation (LOQ) were 100 ng/mL for amphetamine/methamphetamine, 1.5 ng/mL for THC acid, 30 ng/mL for benzoylecgonine, 60 ng/mL for opiates, 80 ng/mL for barbiturates, 60 ng/mL for benzodiazepines, and 2.5 ng/mL for PCP.

Linearity study

Quality-control (QC) samples were purchased and were those that were in use at Kroll Laboratory Specialists (Gretna, LA). Each quality control sample was prepared in pooled, filtered human urine with < 1% sodium azide determined to be drug free by GC–MS. Sources of QC material were CEDIA DAU THC metabolite calibrators at 50 and 100 ng/mL; Multi-Drug Control Set, Multi-Drug Calibrator Set, and Specialty Control Set (Microgenics, Fremont, CA); and Multi-Drug Urine Calibrators and Controls (Diagnostic Reagents, Sunnyvale, CA) for methamphetamine, benzoylecgonine, carboxy-THC, morphine, PCP, secobarbital, and oxazepam. Amphetamine and amitriptyline quality controls were prepared from stock standards (Cerilliant, Austin, TX). Dilutions were prepared to 25%, 50%, 75%, 100%, 125%, and 150% of the cutoffs as described. Drug-free synthetic urine, currently in use in the laboratory, was used for all dilutions.

LOD

To determine the lowest concentration at which the drug could be detected, we diluted QC material to determine the LOD of the assay for each analyte. We used replicates of 5 and defined the LOD as any of the 5 results that produced a positive result. Because the reagent strips contain immobilized drug conjugate, we examined the possibility of contamination of the reagent strips (carryover due to reagent) by exposing the cups to drug-free urine for 30 h and then analyzing the urine by GC–MS at the LOD.

Negative/positive differentiation

In order to assess the ability of the assay to differentiate positive and negative samples, known drug-free negative and known positives (fortified with drug at 125% of the cutoff) were analyzed. Replicates of 20 tests were performed.

Cross-reactivity study

Common structural analogues (e.g., sympathomimetic amines) or compounds with some historical significance (e.g., ibuprofen) were chosen for each drug class to test the specificity of the antibody. The following sympathomimetic amines were chosen because of their structural similarity with amphetamine and methamphetamine: diphenhydramine, ephedrine, pseudoephedrine, D-methamphetamine, L-methamphetamine, methylphenidate, methylenedioxymethamphetamine (MDMA), methylenedioxyethylamphetamine (MDEA), methylenedioxyamphetamine (MDA), phenethylamine, phentermine, mephentermine, and phenylpropanolamine. The opiate cross-reactivity study included synthetic opiates, naturally occurring opiates, and some metabolites such as hydromorphone, hydrocodone, mono-acetylmorphine, codeine, oxycodone, tramadol, meperidine, nor-meperidine, nor-morphine, norcodeine, and buprenorphine. The benzodiazepine cross-reactivity study included commonly prescribed benzodiazepines and their metabolites such as estazolam, flunitrazepam, nordiazepam, oxazepam, lorazepam, triazolam, α-hydroxyalprazolam, 2-hydroxyethylflurazepam, prazepam, and nitrazepam. The common structural analogues of 11-nor-^{9}-THC-9-carboxylic acid, including 11-nor-^{8}-THC-9-carboxylic acid, cannabinol, and cannabidiol, were chosen as well as ibuprofen, which is commonly included on NLCP proficiency tests. In the benzoylecgonine assay, other cocaine metabolites ecgonine and ecgonine methyl ester were used as well as compounds with similar sounding names to cocaine (e.g., lidocaine, tetracaine, or procaine) that donors claim may have caused their positive test. The commonly occurring barbiturates such as butalbital, pentobarbital, amobarbital, phenobarbital, and allobarbital were chosen to challenge the barbiturate assay. The commonly occurring antidepressants amitriptyline, nordoxepein, trimipramine, and protriptyline as well as common phenothiazines chlorpromazine and promethazine were chosen to evaluate the assay. Cross-reactivity at a variety of concentrations of structural analogue were used to show concentrations which resulted in positive or negative results. Stock standards were obtained as methanolic stock standards or prepared from powder in methanol (Cerilliant; Isotec, Miamisburg OH; Sigma Chemical Company, St. Louis, MO; and Aldrich, Milwaukee, WI) and dilutions prepared in synthetic drug-free urine.

Urine specimens

Human urine specimens consisted of volunteer samples or donor samples submitted to Laboratory Specialists Incorporated (Gretna, LA). The TCA donor samples were obtained from Charity Hospital (New Orleans, LA). Samples were selected at random from those containing adequate volume (30 mL) for the required number of trials. Putative negative specimens (N = 50) were chosen from donor samples previously determined to be negative by laboratory immunoassay. Immunoassay positive aliquots (N = 10 for each drug) were taken from specimens submitted for GC–MS analysis. These frozen specimens were retrieved from storage at –20°C.

Testing

All testing was performed by one of the authors (EHT) to prevent variation in technique and to simulate use of the device 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

Linearity challenge

The results of the linearity challenge are shown in Table I. All analytes were positive for all five replicates at or above the cutoff with the exception of opiates (morphine), which was positive in three out of five replicates at the cutoff. At 75% of the cutoff, all replicates of each analyte were positive with the exception of morphine. This indicated a greater degree of sensitivity (greater ability to detect a positive result) at values below the cutoff. This may be advantageous to detect a greater number of positives when compared with the traditional laboratory immunoassay, indicating that the cutoff is actually lower that the manufacturer's stated value. At 50% of the cutoff, all replicates for barbiturates, cocaine metabolite, methamphetamine, and tricyclic antidepressants were positive, and all replicates at 50% of the cutoff for benzodiazepines, opiates, and carboxy-THC were negative. Amphetamine and PCP were mixed (two positive and three negative) at 50% of the cutoff. Only barbiturates were positive at 25% of the cutoff, and all of the remaining analytes were negative for all five replicates. All analytes were negative for all replicates in drug-free urine.

Table I. Linearity of DrugCheck® 9 Test Cup*

Analyte	Percent of Cutoff						
	0%	25%	50%	75%	100%	125%	150%
Amphetamine	–[†]	–	2+/3–[‡]	+	+	+	+
Barbiturates (Secobarbital)	–	+[§]	+	+	+	+	+
Benzodiazepines (Oxazepam)	–	–	-	+	+	+	+
Cocaine metabolite (Benzoylecgonine)	–	–	+	+	+	+	+
Methamphetamine	–	–	+	+	+	+	+
Opiates (Morphine)	–	–	–	–	3+/2–[#]	+	+
PCP	–	–	2+/3–	+	+	+	+
THC-COOH	–	–	-	+	+	+	+
Tricyclic antidepressants (Amitriptyline)	–	–	+	+	+	+	+

* N = 5 challenges per level.
[†] All 5 results were negative (–).
[‡] Indicates 2 (+) and 3 (–) of 5 challenges.
[§] All 5 results were positive (+).
[#] Indicates 3 (+) and 2 (–) of 5 challenges.

Negative/positive differentiation

Results for 20 replicates of drug-free human urine are shown in Table II and results for 20 replicates of known positive specimens fortified at 125% of the cutoff are shown in Table III. Precision was excellent with complete agreement on all replicates. There were no false positives and no false negatives.

Negative donor samples (parallel study)

The results of testing donor samples negative by laboratory immunoassay are shown in Table IV. Visible lines indicating negative test results were very clear and well defined. There was excellent correlation between the POC device and the laboratory

Table II. Analysis of Drug-Free Urine with the DrugCheck 9 Test Cup

Analyte	Negative*	Positive
Amphetamine	20	0
Barbiturates	20	0
Benzodiazepines	20	0
Cocaine metabolite	20	0
Methamphetamine	20	0
Opiates	20	0
PCP	20	0
THC-COOH	20	0
Tricyclic antidepressants	20	0

* N = 20. Specimens contained drug-free human urine.

Table III. Analysis of Positive Specimens with the DrugCheck 9 Test Cup

Analyte	Negative	Positive*
Amphetamine	0	20
Barbiturates	0	20
Benzodiazepines	0	20
Cocaine metabolite	0	20
Methamphetamine	0	20
Opiates	0	20
PCP	0	20
THC-COOH	0	20
Tricyclic antidepressants	0	20

* N = 20. Specimens contained drug at 125% of cutoff.

immunoassay; however, the POC device identified two positive donor samples for cocaine metabolite that were confirmed by GC–MS for benzoylecgonine at 77 and 114 ng/mL. There was one discrepancy for a specimen negative by laboratory immunoassay and positive POC for benzodiazepines which may have had α-hydroxyalprazolam present. The ions were present on GC–MS with selected ion monitoring (SIM) at the correct retention time; however, the ion ratios were outside of the acceptable range of ± 20% of the calibrator because the concentration approached the LOD of the GC–MS assay.

Positive donor samples (parallel study)

The results of testing donor samples positive by laboratory immunoassay are shown in Table V. Specimens were chosen at random from laboratory positives among a wide concentration range in order to adequately challenge the assay. There was agreement for all analytes except for two opiates that were shown to have very low concentrations of total morphine by GC–MS at 346 and 351 ng/mL, respectively. The POC assay showed a positive with morphine glucuronide at 400 ng/mL (17). Because the majority of morphine is metabolized to morphine conjugates, for example, morphine glucuronide, this finding is consistent with the POC cross-reactivity of morphine glucuronide and LOD of free morphine experimentally determined to be 300 ng/mL.

Table IV. Comparison of Immunoassay Negative Donor Samples

Analyte	Negative	Positive
Amphetamine	50	0
Barbiturates	50	0
Benzodiazepines	49	1*
Cocaine metabolite	48	2†
Methamphetamine	50	0
Opiates	50	0
PCP	50	0
THC-COOH	50	0
Tricyclic antidepressants	50	0

* α-Hydroxyalprazolam may be present at a very low concentration. Ions were present on GC–MS.
† Benzoylecgonine confirmed by GC–MS at 77 ng/mL and 114 ng/mL.

Table V. Comparison of Immunoassay Positive Donor Samples*

Analyte	Negative	Positive
Amphetamine	0	10
Barbiturates	0	10
Benzodiazepines	0	10
Cocaine metabolite	0	10
Methamphetamine	0	10
Opiates	2†	8
PCP	0	6
THC-COOH	0	10
Tricyclic Antidepressants	0	10

* $N = 10$ positive donor samples per analyte except PCP where $N = 6$.
† These two samples had very low levels of total morphine (346 ng/mL and 351 ng/mL).

LOD

Because the linearity of the assay extended below the cutoff as described, we further diluted QC material to determine the LOD of the assay for each analyte. Table VI showed the LOD which we defined as any of the five results that produced a positive result.

The two donor samples from Table IV that showed benzoylecgonine at 77 and 114 ng/mL were well below the stated cutoff of 300 ng/mL, and these data are consistent with the extended linearity of the assay with an LOD determined experimentally at 112 ng/mL (Table VI).

When the reagent strips were exposed to drug-free urine for 30 h, analysis of the urine by GC–MS at the LOD verified the absence of reagent contamination

Table VI. Limit of Detection of the DrugCheck® 9 Test Cup

Analyte	LOD*
Amphetamine	500 ng/mL
Barbiturates (Secobarbital)	75 ng/mL
Benzodiazepines (Oxazepam)	225 ng/mL
Cocaine metabolite (Benzoylecgonine)	112 ng/mL
Methamphetamine	375 ng/mL
Opiates (Morphine)	300 ng/mL
PCP	12 ng/mL
THC-COOH	31 ng/mL
Tricyclic antidepressants (Amitrityline)	500 ng/mL

* Replicates of five samples were run. LOD was defined as any of the five replicates that resulted in a positive test.

Table VII. Concentrations (ng/mL) of Common Structural Analogues and Cross-Reactivity with the Amphetamine Assay

Compound	Negative	Positive
Diphenhydramine	100,000	N/A*
Ephedrine	1,000,000	N/A
Pseudoephedrine	1,000,000	N/A
D-Methamphetamine	50,000	N/A
L-Methamphetamine	50,000	N/A
Methylphenidate	100,000	N/A
MDMA	50,000	N/A
MDEA	50,000	N/A
MDA	500	1000
Phenethylamine	25,000	50,000
Phentermine	500	1000
Mephentermine	10,000	100,000
Chloroquine	1,000,000	N/A
Phenylpropanolamine	100,000	N/A

* N/A = not applicable. No interference was observed.

Cross-reactivity

A method validation protocol requires the evaluation of cross-reactive substances. Amphetamines are commonly known to show the highest rate of immunoassay-positive, GC–MS-negative results in the laboratory. Table VII shows the cross-reactivity of common structural analogues with amphetamine with the cup. Ephedrine, pseudoephedrine, and chloroquine were found to produce no interference at 1,000,000 ng/mL. Diphenhydramine, phenylpropanolamine, and methylphenidate produced no interference at 100,000 ng/mL. D-Methamphetamine, L-methamphetamine, MDMA, and MDEA showed no interference at 50,000 ng/mL, indicating a very specific antibody to amphetamine. Interference was observed at low concentrations of phentermine with a positive result at 1000 ng/mL, and mephentermine showed a positive at 100,000 ng/mL. The designer drug MDA showed a positive result at 1000 ng/mL. Phenethylamine produced a positive result at 50,000 ng/mL and a negative result at 25,000 ng/mL.

Table VIII shows the cross-reactivity of common structural analogues with methamphetamine. Ephedrine did not interfere at 1,000,000 ng/mL. The cup produced a positive result at 100,000 ng/mL and a negative result at 50,000 ng/mL of pseudoephedrine. Phentermine, phenylpropanolamine, diphenhyramine, and methylphenidate showed no interference at 100,000 ng/mL. The designer drug MDMA produced a positive result at 1000 ng/mL, and L-methamphetamine was positive at 5000 ng/mL. The antibody is very specific to methamphetamine because D-amphetamine and MDA produced a negative result at 50,000 ng/mL. MDEA was positive at 50,000 ng/mL but negative at 10,000 ng/mL.

Table IX shows the cross-reactivity of common structural analogues with the opiate (morphine) assay. Oxycodone, oxymorphone, tramadol, meperidine, nor-meperidine, nor-morphine, nor-codeine, and buprenorphine showed no interference at 50,000 ng/mL. Codeine produced a positive result at 300 ng/mL, and hydromorphone and hydrocodone produced a positive result at 5000 ng/mL. Monoacetylmorphine, the heroin metabolite, showed a positive at 2500 ng/mL.

A large number of benzodiazepines are detectable (Table X). Estazolam, nordiazepam, oxazepam, lorazepam, triazolam, clonazepam, temazepam, α-hydroxyalprazolam, and nitrazepam showed a positive result at or below the cutoff. Flunitrazepam and prazepam produced a positive result at 500 ng/mL.

Carboxy-THC was unaffected by ibuprophen at 1,000,000 ng/mL and also by cannbinol and cannabidiol at 50,000 ng/mL (Table XI). The analogue, 11-nor- ^{8}-THC-9-carboxylic acid, showed positive at 200 ng/mL indicating a high degree of specificity compared to the target analyte, 11-nor- ^{9}-THC-9-carboxylic acid which was positive as low as 31 ng/mL (Table VI).

No interference was observed in the cocaine metabolite (benzoylecgonine) assay from the other metabolites ecgonine or ecgonine methyl ester at 50,000 ng/mL (Table XII). No interference from lidocaine, tetracaine, or procaine at 1,000,000 ng/mL was present.

Table XIII shows the cross-reactivity of common structural

Table VIII. Concentrations (ng/mL) of Common Structural Analogues and Cross-Reactivity with the Methamphetamine Assay

Compound	Negative	Positive
Diphenhydramine	100,000	N/A*
Ephedrine	1,000,000	N/A
Pseudoephedrine	50,000	100,000
D-Amphetamine	50,000	N/A
L-Methamphetamine	2000	5000
Methylphenidate	100,000	N/A
MDMA	500	1000
MDEA	10,000	50,000
MDA	50,000	N/A
Phenethylamine	25,000	50,000
Phentermine	100,000	N/A
Mephentermine	5000	10,000
Chroloquine	100,000	1,000,000
Phenylpropanolamine	100,000	N/A

* N/A = not applicable. No interference was observed.

Table IX. Concentrations (ng/mL) of Common Structural Analogues and Cross-Reactivity with the Opiate Assay

Compound	Negative	Positive
Hydromorphone	2000	5000
Hydrocodone	2000	5000
Monoacetylmorphine	1000	2500
Codeine	250	300
Oxycodone	50,000	N/A*
Oxymorphone	50,000	N/A
Tramadol	50,000	N/A
Meperidine	50,000	N/A
Nor-Meperidine	50,000	N/A
Nor-Morphine	50,000	N/A
Nor-Codeine	50,000	N/A
Buprenorphine	50,000	N/A

* N/A = not applicable. No interference was observed.

Table X. Concentrations (ng/mL) of Common Structural Analogues and Cross-Reactivity with the Benzodiazepine Assay

Compound	Negative	Positive
Estazolam	N/A*	100
Flunitrazepam	300	500
Nordiazepam	100	300
Oxazepam	100	300
Lorazepam	100	300
Triazolam	100	300
Clonazepam	100	300
Temazepam	100	300
α-Hydroxyalprazolam	N/A	100
2-Hydroxy-ethyl-flurazepam	100	300
Prazepam	300	500
Nitrazepam	100	300

* N/A = not applicable. Lower concentations were not necessary.

analogues with the barbiturates (secobarbital) assay. Pentobarbital, amobarbital, phenobarbital, and allobarbital produced a positive result at or below the cutoff. Butalbital showed a positive at 500 ng/mL.

In the tricyclic antidepressant assay, amitriptyline and nordoxepin produced a positive test at 300 ng/mL, well below the cutoff of 1000 ng/mL (Table XIV). Trimipramine and protriptyline produced a positive at 2500 ng/mL and 5000 ng/mL, respectively. The phenothiazines, chlorpromazine and promethazine showed interference with a positive result at 25,000 ng/mL and 50,000 ng/mL, respectively.

In addition, we assessed the cross-reactivity of dextromethorphan in the PCP assay. No interference was observed at 100,000 ng/mL.

Discussion

Current laboratory-based urine drug testing involves local specimen collection, careful chain of custody procedures for the sample during shipment to the laboratory, and progress through testing, which has been refined during the past decade to minimize procedural and administrative errors. In theory, POC drug testing offers the advantages of speed and reduced cost over in-laboratory testing. As DTAB reviews the various alternate matrices with possible implementation for DOT testing in the future (15,16), each method will have to be validated by means of a standard protocol as is currently done for a new immunoassay method in a SAMHSA-certified laboratory (18). The POC device will need to be validated by either the manufacturer (as is currently done in the breath alcohol model) or validated by the end user which seems unlikely. We chose to adapt the NLCP method validation protocol (18) as is currently done in a certified laboratory in order to validate the DrugCheck 9 Cup. A literature review did not yield information regarding similar extensive method validation (1–14) for other POC devices, although QC performance challenges around the stated cutoff and some cross-reactivity studies have been done (11).

In this study, the Drug Check 9 Cup was evaluated according to the NLCP protocol which requires a validation study to include 1. linearity around the cutoff by analyzing a negative calibrator and 6–8 concentrations with replicates of five using the cutoff concentration as a mid-range point; 2. precision using the replicate data given; 3. accuracy; 4. positive/negative sample analysis by analyzing a combination of negative urine samples and negative urine samples fortified with known amounts of drug with a minimum of 10 replicates; 5. a parallel study using at least 100 aliquots of previously assayed donor samples; and 6. specificity by evaluation of the assay's performance when challenged with compounds chemically similar to the assay of interest at concentrations equivalent to those investigated in a previously validated immunoassay.

In the linearity challenge, the DrugCheck Cup was positive at or above the cutoff for all analytes except opiates which showed mixed results at the cutoff. All analytes (except opiates) at 25% below the cutoff were positive, indicating that the actual cutoff for these analytes is at or below 75% of the cutoff which some might consider a desirable error in testing. The linearity for some analytes, for example, barbiturates, cocaine metabolite, methamphetamine, and tricyclic antidepressants was well below the manufacturer's stated cutoff, which will identify a greater number of positive results. This indicated a higher degree of

Table XI. Concentrations (ng/mL) of Common Structural Analogues and Cross-Reactivity with the Carboxy-THC Assay

Compound	Negative	Positive
Ibuprofen	1,000,000	N/A*
Cannabinol	50,000	N/A
Cannabidiol	50,000	N/A
11-Nor- ^{8}-THC-9-carboxylic acid	100	200

* N/A = not applicable. No interference observed.

Table XII. Concentrations (ng/mL) of Common Structural Analogues and Cross-Reactivity with the Benzoylecgonine Assay

Compound	Negative	Positive
Ecgonine	50,000	N/A*
Ecgonine methyl ester	50,000	N/A
Lidocaine	1,000,000	N/A
Tetracaine	1,000,000	N/A
Procaine	1,000,000	N/A

* N/A = not applicable. No interference observed.

Table XIII. Concentrations (ng/mL) of Common Structural Analogues and Cross-Reactivity with the Barbiturate Assay

Compound	Negative	Positive
Butalbital	300	500
Pentobarbital	N/A*	100
Amobarbital	N/A	100
Phenobarbital	N/A	100
Allobarbital	100	300

* N/A = not applicable. Lower concentrations were not necessary.

Table XIV. Concentrations (ng/mL) of Common Structural Analogues and Cross-Reactivity with the Tricyclic Antidepressant Assay

Compound	Negative	Positive
Amitriptyline	100	300
Nordoxepin	100	300
Trimipramine	1000	2500
Protriptyline	1000	5000
Chlorpromazine	10,000	25,000
Promethazine	25,000	50,000

sensitivity (ability to identify a positive), because the POC device showed an extended linearity below the manufacturer's stated cutoff values.

The interpretation is subjective; the presence of a line, even a faint line, is interpreted as a negative result, as described in the manufacturer's package insert (17). Although the assay has a certain time window to read the result (usually 5–10 min), some assays will change over time and a line can appear after the time window stated by the manufacturer. The rose-pink lines were very distinct (a negative result) in drug-free urine and at very low concentrations of drug but were absent (a positive result) at or above the cutoff, which is highly desirable. Interpretation was more difficult with very faint lines in situations in which drug was present but below the cutoff. Consistency in reading the visual rose-pink line is necessary to ensure correct results.

The parallel study with donor samples that had been previously screened by the laboratory showed excellent agreement with the laboratory immunoassay with 123 out of 126 in agreement. The POC device identified two cocaine metabolite positives that screened negative by laboratory immunoassay but yet confirmed by GC–MS, although at low concentrations, which was consistent with the linearity challenge. The additional discrepant result was a benzodiazepine positive on the POC device that may have α-hydroxyalprazolam present but could not be confirmed by GC–MS due to its low quantitative value. Two opiate results that were within 20% of the cutoff were missed due to the presence of morphine conjugates.

The increased sensitivity may be of significant value in a criminal justice or a clinical setting but may produce a greater number of positives in the employment setting because the POC device can identify positives below the current SAMHSA cutoffs. There are practical implications of false-positive immunoassay results due to cross-reaction with structural analogues. Such results are invisible to the end user of the conventional laboratory drug test because the initial positive result is subsequently refuted by GC–MS; the medical review officer or employer sees only the confirmed negative. With immediate test results, the on-site tester bears the burden of recognizing that interferences exist and that an initial result is not the end result. The antibody used in this device demonstrated improved specificity compared to earlier POC devices (11). For example, methamphetamine did not interfere in the amphetamine assay and vice versa. Only phentermine caused a significant interference with the amphetamine test and mephentermine with the methamphetamine assay, a considerable improvement over earlier versions of POC test devices which showed interferences due to ephedrine at 1,000,000 ng/mL and phenylpropanolamine at 10,000 ng/mL (11). The designer drugs MDA and MDMA would be correctly identified because the cross-reactivity showed positive results at the cutoff of 1000 ng/mL of amphetamine and methamphetamine, respectively. The opiate assay was specific for codeine and morphine with some cross-reactivity for hydrocodone and hydromorphone as would be expected. The synthetic opiate, oxycodone, which is commonly abused today, was not detected at 50,000 ng/mL. The assay for benzodiazepines and barbiturates detected all of the common drugs at or below the cutoff which is certainly advantageous in a clinical setting.

When the on-site test is "successful" (correctly detects absence of illicit drugs), the employer/criminal justice system/health care worker benefits from immediate results at a cost comparable to or lower than that of a conventional laboratory drug test. We would strongly caution employers that the GC–MS confirmation is a necessary component of the overall testing process. However, if an on-site test is positive due to cross-reactive over-the-counter cold medications or other interferences, the employer must wait for confirmation of the initial result by GC–MS analysis at the laboratory. Because of increased specificity due to decreased cross-reactivity of over-the-counter medications of this POC device, there is less likelihood that the specimen will be needlessly sent to the laboratory for confirmation.

In a laboratory, there are requirements for initial test method validation, a standard operating procedure manual (SOP), quality control (QC), quality assurance (QA), proficiency/certification training of analysts, proficiency testing, security, chain of custody (COC), confirmation by GC–MS procedures, documentation review, recordkeeping, and reporting procedures. This study used a widely used POC device and provides an example of a standard method validation mimicking the NLCP laboratory protocol. Clearly, the listed components are essential to provide POC testing that is equivalent to laboratory-based testing. SAMHSA, through DTAB, is currently reviewing the requirements in order for a POC device to comply with federal requirements and this study provides data to evaluate the accuracy and feasibility of use.

In summary, the DrugCheck 9 Cup offers a rapid result with excellent correlation to results of donor samples analyzed in a laboratory with 123 out of 126 (97.6%) consistent with the immunoassay or GC–MS result; however, our study shows that the testing cutoffs for the DrugCheck 9 cup are below those in use in a SAMHSA-certified laboratory. The cup's extended linearity below the cutoff will generate addition positives. This may be desirable in the criminal justice or clinical setting to identify more positives; however, one should be aware of this in a workplace setting. Even with the improved antibody specificity to minimize undesirable cross-reactive substances, the manufacturer strongly recommends that presumptive positive specimens be confirmed by GC–MS just as is currently done in the laboratory.

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