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A Comparison of ONTRAK TESTCUP™, Abuscreen ONTRAK®, Abuscreen ONLINE®, and GC/MS Urinalysis Test Results*

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ABSTRACT: This study was designed to compare results obtained from two separate on-site drug testing kits (ONTRAK TESTCUP and Abuscreen ONTRAK) with those obtained from laboratory based immunoassay and GC/MS. Abuscreen ONLINE immunoassay was used to select 250 negative samples and 100 presumptive-positive samples each for cocaine/metabolites, opiates and cannabinoids. Presumptive-positive samples were selected if the immunoassay response was ⩾300 ng/mL for cocaine/metabolites (BZE), ⩾300 ng/mL for opiates or ⩾50 ng/mL for cannabinoids (THC-COOH). GC/MS was used to confirm that each selected sample contained ⩾150 ng/mL BZE, ⩾300 ng/mL morphine/codeine or ⩾15 ng/mL THC-COOH.

TESTCUP results had a 100% agreement with GC/MS and a >99% agreement with ONLINE when testing negative samples. The agreement between TESTCUP and ONLINE results for samples containing opiates was 100%. Results of testing samples containing BZE with TESTCUP demonstrated a 98% agreement with both GC/MS and ONLINE. Both discrepant samples contained BZE at concentrations ⩽300 ng/mL. The least agreement between TESTCUP and ONLINE results was found when testing samples containing THC-COOH. The agreement with ONLINE and GC/MS was 92% and all discrepant samples had GC/MS determined THC-COOH concentrations less than 50 ng/mL. A 100% agreement was obtained between expected and recorded TESTCUP results for QC samples fortified to contained BZE, morphine or THC-COOH at concentrations within 120% of the screening cutoffs.

ONTRAK had a 100% agreement with both GC/MS and ONLINE when testing negative samples and samples that contained opiates. ONTRAK had a 91% agreement with GC/MS and ONLINE for testing of samples that contained BZE. The least agreement between ONTRAK and ONLINE results was found when testing samples that contained THC-COOH. The agreement was 89%, however, all discrepant samples contained GC/MS concentrations of THC-COOH less that the 50 ng/mL cutoff. With ONTRAK, a 100% agreement was obtained between expected and recorded results on QC samples that contained morphine or THC-COOH and a 97.7% agreement was obtained between expected and recorded results on QC samples that contained BZE.

KEYWORDS: forensic science, forensic toxicology, on-site drug testing, GC/MS, immunoassay

Illegal drug use continues to present major health, economic and legal problems in the U.S. Urinalysis drug testing remains the most popular and cost effective method of identifying drug users and drug abusers. Laboratory based immunoassay tests have become very popular for both clinical and forensic urinalysis drug testing. Despite early concerns about the accuracy and specificity of these tests, they have gained wide acceptance (1). In clinical laboratories, they are used to test urine collected from patients suspected of drug over-doses and to ensure compliance with drug treatment therapies. In medicolegal laboratories, immunoassay tests are used to screen urine collected from prisoners, parolees, employees, impaired drivers, fatally injured drivers and in medical examiner investigations. Laboratory based immunoassay tests are easy to performed and are readily automated, however, they have several disadvantages. Sophisticated instrumentation is needed to automate testing and considerable technical expertise is required to reliably perform the tests. A permanent laboratory must often be constructed to house the instrumentation. The turn-around-time from specimen collection to receipt of the drug test results using laboratory based immunoassay tests often exceeds 24 hours. These time delays may delay treatment of clinical patients, slow processing of suspected criminals and be costly to employers due to lost wages, delays in hiring and lower productivity.

Recently, more expedient urinalysis drug testing technologies have been introduced. These are on-site urinalysis drug testing kits. The kits are commercially available, immuno-chemistry based, and do not require sophisticated instrumentation, a permanent laboratory or extensive training of personnel. Several kits are currently described in the literature TRIAGE (2–4), ONTRAK (5–6), EZ-SCREEN (8), AccuPinch (9), Mach IV (10); Verdict (10), AbuSign (10), Biosign (10), I.D.Block (10) and Testcup (11). The kits have been advocated for drug screening in clinical settings, the criminal justice system, nuclear power generating plants, offshore oil drill platforms, commercial trucking and highway safety.

The objective of the study presented here was to compare results obtained from two separate on-site drug testing kits (ONTRAK TESTCUP and Abuscreen ONTRAK) with those obtained from a laboratory based immunoassay (Abuscreen ONLINE) and gas chromatography mass spectrometry (GC/MS). To achieve this objective, human urine samples were tested by these different tech-

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nologies for cocaine/metabolites, morphine/codeine and cannabinoids.

Materials and Methods

Abuscreen ONLINE was selected as the laboratory based immunoassay (Roche Diagnostics Systems, Inc., Branchburg, NJ 08876-1760). This testing procedure relies on the kinetic interaction of microparticles in solution (KIMS) to detect drugs of abuse in urine (12). Using ONLINE testing, Northwest Toxicology (NWT), Inc., (Salt Lake City, UT) selected 250 negative samples and 100 presumptive-positive samples each for cocaine/metabolites, opiates and cannabinoids. The ONLINE analyses were performed with an Hitachi 717 (Boehringer Manhiem Diagnostics, Indianapolis, IN) automated analyzer. Presumptive-positive samples were selected if the immunoassay response was ⩾300 ng/mL for cocaine/metabolites, ⩾300 ng/mL for opiates or ⩾50 ng/mL for cannabinoids (13,14). Also at NWT, GC/MS with deuterium labeled internal standards, selected ion monitoring and ion ratios was used to confirm that each selected sample contained benzoylecgonine (BZE), morphine/codeine or 11-nor-delta-9-THC-carboxylic acid (THC-COOH). Samples were categorized as positive and tested with the on-site kits if they contained ⩾300 ng/mL of total morphine and codeine, ⩾150 ng/mL of benzoylecgonine, or ⩾15 ng/mL of hydrolyzed THC-COOH by GC/MS. GC/MS analysis was not performed on negative samples. The 1 to 2 mL sample aliquot that remained from the ONLINE testing was transferred to the Center for Human Toxicology (CHT) (University of Utah, Salt Lake City, UT) for the on-site testing.

The ONTRAK TESTCUP immuno-reaction is based on competition between drug in the urine and a drug conjugate immobilized on a testing membrane for limited antibody coated onto colored microparticles (15). If drug is present in the tested urine, the drug conjugate is inhibited from reacting with the antibody and no color is observed in the test visualization area. If the tested urine is negative, the drug conjugate combines with the antibody and a blue color develops in the testing area. The cups are designed to detect ⩾300 ng/mL of BZE, ⩾300 ng/mL of morphine and ⩾50 ng/mL THC-COOH. Each cup tests for the three drug classes concurrently, therefore, each sample received from NWT was analyzed for the all three drug groups regardless of ONLINE category. All samples were tested by the on-site kit in batches of approximately 50 samples plus 10% negative and positive quality control samples. Tests were performed as soon as logistically possible following identification by ONLINE. Particular attention was placed on timely analyses of THC-COOH selected samples to minimize the possible effects of drug degradation (16). To perform a test following a urine collection from a donor, the manufacturer's procedure instructs the analyst to turn the TESTCUP lid to the test position and tilt the cup allowing the specimen to fill the sample reservoir. This initiates the immuno-reaction (15). This procedure was modified to accommodate the limited sample volumes available in this study. A pipet was used to add approximately 400 μL of urine directly to the "sample reservoir."

Unlike TESTCUP, each Abuscreen ONTRAK kit tests for a single drug class (17). Each kit is designed to detect ⩾300 ng/mL of BZE, ⩾300 ng/mL of morphine or ⩾50 ng/mL of THC-COOH. Like ONLINE, ONTRAK test kits are based on competition between drug in the urine and a latex-drug conjugate reagent supplied in the kit for limited antibody binding sites. If drug is present in the tested urine, the latex-drug particles remain monomeric and the test mixture appears "milky." If the tested urine is negative, the latex-drug particles combine with the antibody and a visible aggregate forms. Samples were analyzed according to the manufacturer's recommendations for all three drug groups regardless of ONLINE category. All samples were tested by the on-site kits in batches of approximately 50 samples plus 10% quality control samples. Tests were performed as soon as logistically possible following identification by ONLINE. Particular attention was placed on analyzing THC-COOH selected samples to minimize the possible effects of drug degradation (16).

Samples that screened drug free by ONLINE were placed in a "Negative Category." Samples that screened positive by ONLINE and confirmed positive by GC/MS were placed in the appropriate "Cocaine Positive Category," "Opiate Positive Category" or "THC-COOH Positive Category." Some samples screened and confirmed positive for two drug groups and were included in both categories.

Samples that screened positive by TESTCUP or ONTRAK for drugs not detected by ONLINE required additional GC/MS confirmation. For example, if a sample tested positive for opiates by ONLINE and negative for BZE and THC-COOH, then it was placed in the Opiate Positive Category. However, when tested by TESTCUP or ONTRAK, if that sample tested positive for BZE or THC-COOH, then a GC/MS confirmation for the additional drug was performed. BZE and opiate presumptive positive results were confirmed at CHT by positive ion chemical ionization mass spectrometry and DHHS cutoffs were not observed (18,19). THC-COOH presumptive positive results were confirmed at CHT by negative ion chemical ionization mass spectrometry (20).

Results

Negative Category

Two hundred and fifty negative samples were selected to challenge the ONTRAK and TESTCUP test kits. These samples tested less than the DHHS cutoffs for cannabinoids, cocaine metabolites and opiates by ONLINE. Table 1 shows the results of this testing. Of 750 tests performed with TESTCUP, only 1 Negative Category sample tested presumptively positive. This sample was positive for cannabinoids. Compared to ONLINE, this equated to a 99.87% agreement for all drug tests and a 99.6% agreement for cannabinoids. However, this sample was analyzed by GC/MS and it contained 13.5 ng/mL of THC-COOH. Therefore, the actual unconfirmed positive rate (vs. GC/MS) was 0.0%. The Table also shows that of 750 tests performed with ONTRAK, no samples tested presumptively positive for any of the three drug classes. Therefore, the agreement rate for ONLINE negative samples was 100% and there were no unconfirmed positive samples.

Opiate Positive Category

One hundred opiate positive samples were selected to challenge the TESTCUP and ONTRAK. These samples tested positive for

TABLE 1—*Results: negative category samples.*

	TESTCUP	ONTRAK
Number of Samples	250	250
Number of Tests	750	750
Discrepancies	1*	0
Percent Agreement	99.9%	100.0%
Percent Agreement (THC-COOH)	99.6%	100.0%
Unconfirmed Positive Rate	0.0%	0.0%

*Sample contained 13.5 ng/mL of THC-COOH.

opiates by ONLINE and contained a combined codeine and morphine concentration of ⩾300 ng/mL by GC/MS. The mean, standard deviation and range of the GC/MS codeine concentrations respectively were 6,684 ng/mL, 16,977 ng/mL and 0 to 96,578 ng/mL. The mean, standard deviation and range of the GC/MS morphine concentrations respectively were 1,954 ng/mL, 4,207 ng/mL and 0 to 27,847 ng/mL. Table 2 shows all 100 of the 100 samples (100%) that tested positive by ONLINE for opiates also tested positive by TESTCUP and ONTRAK.

Cocaine Positive Category

One hundred samples that tested positive for cocaine metabolites by ONLINE and contained ⩾150 ng/mL of BZE by GC/MS were selected to challenge TESTCUP and ONTRAK. The mean, standard deviation and range of the GC/MS BZE concentrations respectively were 18,275 ng/mL, 65,475 ng/mL and 168 to 476,912 ng/mL. Table 2 shows that 98 of the 100 samples (98%) that tested positive by ONLINE were also positive by TESTCUP. The two discrepant samples had GC/MS BZE concentrations of 168 ng/mL and 205 ng/mL. With ONTRAK, 91 of 100 samples (91%) that tested positive by ONLINE were also positive by ONTRAK. The nine discrepant samples had a mean GC/MS BZE concentration of 344.1 ng/mL, a standard deviation of 202.9 ng/mL and a range of 168 ng/mL to 728 ng/mL. The two samples discrepant by TESTCUP also tested negative by ONTRAK.

THC-COOH Positive Category

One hundred samples that tested positive for cannabinoids by ONLINE and contained ⩾15 ng/mL of THC-COOH by GC/MS were selected to challenge TESTCUP and ONTRAK. The mean, standard deviation and range of the GC/MS THC-COOH concentrations respectively were 147, 184, and 18 to 1,295 ng/mL. Table 2 shows that 92 of the 100 samples (92%) that tested positive by ONLINE also tested positive by TESTCUP. The mean GC/MS concentration of the discrepant samples was 29.1 ng/mL, the standard deviation 8.6 ng/mL and the range 21 ng/mL to 46 ng/mL. Eighty-nine of 100 samples (89%) that tested positive by ONLINE also tested positive by ONTRAK. The mean GC/MS concentration of the discrepant samples was 28.7 ng/mL, the standard deviation 5.6 ng/mL, and the range 21 to 36 ng/mL. Six discrepant samples tested negative by both on-site kits. All discrepant samples were re-analyzed by ONLINE.

TABLE 2—*Results: positive category samples.*

	TESTCUP	ONTRAK
Opiate Positive Category		
Number of Samples	100	100
Number of Tests	100	100
Discrepancies	0	0
Percent Agreement	100.0%	100.0%
Cocaine Positive Category		
Number of Samples	100	100
Number of Tests	100	100
Discrepancies	2*	9†
Percent Agreement	98.0%	91.0%
THC-COOH Positive Category		
Number of Samples	100	100
Number of Tests	100	100
Discrepancies	8	11
Percent Agreement	92%‡	89%§

*Samples contained BZE at 168 and 205 ng/mL.
†Mean BZE concentration 344.1 ng/mL, range (168–728 ng/mL).
‡Improved to 94% with ONLINE retesting.
§Improved to 99% with ONLINE retesting.

Additional Tests Category

All ONLINE positive samples were analyzed by both on-site test kits for all three drugs. For example, samples selected as positive challenges for opiates were also tested by both TESTCUP and ONTRAK for cocaine and cannabinoids. These samples provided additional negative and positive challenges for the on-site test kits. In addition, some of these samples tested positive for drugs by TESTCUP and/or ONTRAK that were not detected by ONLINE. Each sample that was positive by TESTCUP or ONTRAK and negative by ONLINE was subjected to GC/MS analysis at CHT to determine if the TESTCUP/ONTRAK result could be confirmed.

Testcup

One Opiate Positive Category sample tested positive for THC-COOH by TESTCUP. The sample did not contain measurable quantities of THC-COOH by GC/MS and, therefore, was an unconfirmed positive result. Three cocaine positive category samples tested positive for THC-COOH by TESTCUP. All had measurable THC-COOH by GC/MS. The concentrations were 12.7, 15.5, and 31.3 ng/mL. One THC-COOH positive category sample tested positive for opiates with TESTCUP. This sample contained only 12.3 ng/mL of codeine by GC/MS and should be considered an unconfirmed positive given a 300 ng/mL cutoff.

Ontrak

One opiate positive category sample tested positive for THC-COOH by ONTRAK. The sample did not contain measurable quantities of THC-COOH by GC/MS and, therefore, was an unconfirmed positive result. One cocaine positive category sample also tested positive for THC-COOH with ONTRAK, did not contained measurable quantities of THC-COOH by GC/MS and was an unconfirmed positive result. Two THC-COOH positive category samples tested positive for opiates by ONTRAK. One sample contained 49 ng/mL of hydrocodone by GC/MS and the other contained 12.3 ng/mL of codeine.

Quality Control Samples

Sixty-four in-house prepared quality control (QC) samples were included in the TESTCUP procedures. Table 3 shows that 18 of the samples were negative. The remaining QC samples contained GC/MS verified concentrations of BZE and morphine or THC-COOH fortified at 120% of the DHHS screening cutoff concentrations. Table 3 shows that 18 of the 18 negative QC samples (100%) tested negative by TESTCUP. Since TESTCUP simultaneously analyzes for all of the drugs, three tests were performed on each sample. Therefore, TESTCUP was subjected to a total of 54 negative challenges with no errors. Twenty-four of 24 (100%) of the QC samples containing BZE and morphine were correctly identified as were all 22 QC samples that contained THC-COOH. During these analyses, an additional 68 ($24 + 22 \times 2$) correct negative test results were recorded.

Each ONTRAK slide tests for a single drug, therefore, the data obtained represent one test challenge/QC sample. All 18 negative

TABLE 3—*Results: quality control samples.*

	TESTCUP	ONTRAK
Negative		
Number of Samples	18	18
Discrepancies	0	0
Percent Agreement	100.0%	100.0%
Opiates		
Number of Samples	24	43
Discrepancies	0	0
Percent Agreement	100.0%	100.0%
BZE		
Number of Samples	24	43
Discrepancies	0	1
Percent Agreement	100.0%	97.7%
THC-COOH		
Number of Samples	22	41
Discrepancies	0	0
Percent Agreement	100.0%	100.0%

(100%), 43 opiate (100%) and 41 THC-COOH (100%) were correctly identified by ONTRAK. The sole QC error was 1 negative result on a sample containing BZE (97.7%).

Comparison of TESTCUP and ONTRAK Results

This section presents a comparison of TESTCUP results to ONTRAK results (Table 4). Of the 250 Negative Category samples, and 1500 combined tests performed with TESTCUP and ONTRAK, only 1 discrepant result was obtained. This sample tested positive for cannabinoids by TESTCUP and negative by ONTRAK. As discussed, the sample contained 13.5 ng/mL of THC-COOH by GC/MS. This error equated to a 0.07% discrepancy rate between TESTCUP and ONTRAK for all tests (1/1500) and a 0.4% (1/250) discrepancy rate for all samples. With positive category samples, there was a 100% agreement for opiate results for the two test kits. Ninety-three (93%) of the cocaine positive category samples gave the same results by both TESTCUP and ONTRAK and all discrepant samples were positive by TESTCUP and negative by ONTRAK. These discrepant samples had a mean GC/MS BZE concentration of 389.1 ng/mL, a standard deviation of 210.0 ng/mL and a range of 192 to 728 ng/mL. There was also a ninety-three (93%) agreement between TESTCUP and ONTRAK for THC-COOH positive category samples. Of the seven discrepant results, five samples were positive by TESTCUP and negative by ONTRAK. These samples had a mean GC/MS THC-COOH concentration of 31.0 ng/mL, a standard deviation of 4.2 ng/mL and a range of 25 to 36 ng/mL. Two discrepant samples were positive by ONTRAK and negative by TESTCUP. These samples had GC/MS THC-COOH concentrations of 26 and 46 ng/mL.

TABLE 4—*Results: TESTCUP vs. ONTRAK.*

	Number of Samples	Total Number of Tests	Discrepancies	% Agreement
Category				
Negative	250	1500	1*	99.6
Opiates	100	200	0	100.0
BZE	100	200	7†	93.0
THC-COOH	100	200	7‡	93.0

*Sample contained 13.5 ng/mL of THC-COOH.

†All samples were positive by TESTCUP and Negative by ONTRAK (mean 389.1 ng/mL) (range 192 to 728 ng/mL).

‡Samples contained a mean of 31.0 ng/mL of THC-COOH (range 25 to 36 ng/mL).

Potential ONLINE Errors (TESTCUP and ONTRAK vs ONLINE)

The discussion above has focused primarily on comparing TESTCUP and ONTRAK results with those obtained by ONLINE and GC/MS. Since the ONLINE test results were used to categorize the samples as positive or negative, the ONLINE results were the standard to which the other tests were compared. However, it seems reasonable to evaluate the data such that TESTCUP and ONTRAK test results be given equal credibility with ONLINE. For example, if a sample tested less than the cutoff for opiates by ONLINE, but positive by TESTCUP and/or ONTRAK and contained a combined opiate concentration $\geqslant$ 300 ng/mL by GC/MS, then perhaps it should not be considered as a testing error by the on-site test kits and the ONLINE test result should be questioned.

No negative category tested positive by both TESTCUP and ONTRAK. No opiate positive category samples tested negative by both TESTCUP and ONTRAK. However, two cocaine positive category samples tested negative by both TESTCUP and ONTRAK. These samples had GC/MS BZE concentrations of 205, and 168 ng/mL respectively. Six THC-COOH positive category tested negative by both TESTCUP and ONTRAK. These samples had a mean GC/MS THC-COOH concentration of 26.8, a standard deviation of 6.2, and a range of 21 to 36 ng/mL.

Discussion

In this study, TESTCUP results had a 100% agreement with GC/MS and a >99% agreement with ONLINE when testing negative samples. When testing positive samples, the most agreement between TESTCUP and ONLINE results was with opiate positive category samples. The agreement with both ONLINE and GC/MS was 100%. Results of testing cocaine positive category samples with TESTCUP demonstrated a 98% agreement with both GC/MS and ONLINE. However, both discrepant samples contained BZE at concentrations < 300 ng/mL TESTCUP cutoff. The least agreement between TESTCUP and ONLINE results was found when testing THC-COOH positive category samples. The agreement with ONLINE and GC/MS was 92%. However, all discrepant samples had GC/MS determined THC-COOH concentrations less than the 50 ng/mL TESTCUP cutoff. These results are not as accurate as those published by Towt, et al., 1995, for TESTCUP (11). These authors found a 100% agreement between TESTCUP and ONLINE results for BZE and THC-COOH and 99% agreement for opiate results.

A 100% agreement was obtained between expected and recorded TESTCUP results for QC samples that contained BZE, morphine or THC-COOH. A major difference between these samples and the donor samples was that the QC samples contained GC/MS verified drug concentrations in excess of the TESTCUP cutoffs. These results are an improvement over those published previously. Towt, et al., 1995, reported a 97, 100, and 98% agreement between QC samples fortified at the same concentrations used in this study (11). Similar results are reported here and by Towt, et al., 1995, of 100% accuracy in testing negative QC samples (11).

ONTRAK had a 100% agreement with both GC/MS and ONLINE when testing negative samples. The most agreement between ONTRAK and ONLINE results was found when testing opiate

positive category samples. The agreement with ONLINE and GC/MS was 100%. Results of testing cocaine positive category samples with ONTRAK had a 91% agreement with GC/MS and ONLINE. However, only four of the nine discrepant samples contained GC/MS determined BZE concentration $\geqslant$ than the 300 ng/mL cutoff. The least agreement between ONTRAK and ONLINE results was found when testing THC-COOH positive category samples. The agreement with ONLINE and GC/MS was 89%. However, all discrepant samples contained GC/MS concentrations of THC-COOH less that the 50 ng/mL cutoff. With ONTRAK, a 100% agreement was obtained between expected and recorded results on QC samples that contained morphine or THC-COOH and a 95.8% agreement was obtained between expected and recorded results on QC samples that contained BZE.

Testing of samples that were included in the additional tests category provided 200 additional negative drug challenges for each drug class to both on-site kits. With TESTCUP, one unconfirmed positive THC-COOH result and one unconfirmed positive opiate result were observed (0.50%). Two unconfirmed positive THC-COOH and opiate results were recorded with ONTRAK (1.00%).

For testing of negative samples, there was essentially a 100% agreement between TESTCUP and ONTRAK (99.93%). The agreement for samples that contained BZE, opiates or THC-COOH was 93, 100, and 93% respectively. These findings are more consistent than those found by comparing either on-site test kit results to results obtained by ONLINE. However, these results are not as good as those reported by Towt, et al., 1995, who found a >99% agreement between TESTCUP and ONTRAK for samples containing BZE, opiates or THC-COOH (11).

A major consideration in the use of any analytical technique is the accuracy of the test. Numerous studies have been published to assess the accuracy and reliability of on-site drug screening test kits (2–4,8,10). The basic design of these studies was similar to the study reported here. On-site test results were compared to test results obtained from one or more alternate methods. However, predicting a positive or negative on-site test result based on an initial laboratory immunoassay test or GC/MS quantitation requires a thorough understanding of all of the methods used and the testing techniques. The study design should ensure that samples are analyzed by each testing technique as contemporaneously as possible to avoid sample and analyte degradation (16). The analyst must understand the reactivity of each immunoassay test to the specific drug or metabolite detected. The QC results obtained with the TESTCUP and ONTRAK demonstrate an additional problem. Clearly, the performance of both TESTCUP and ONTRAK was improved when, unlike donor samples, the urine contained a verified concentration of the drug in excess of the assays published cutoff.

An additional consideration in comparing immunoassay tests is assay variability at the cutoff and is demonstrated by the following example. If a sample that contained 50 ng/mL of immuno-reactive cannabinoids, by a laboratory based screen, were tested multiple times, alternate positive and negative results in equal proportions would be predicted. If a statistically significant-number of analyses were performed, a normal distribution of results would be predicted. During routine laboratory based urinalysis testing, this expected assay variation would also result in samples containing >50 ng/mL of reactive urinary cannabinoids sometimes testing negative and those containing <50 ng/mL of reactive urinary cannabinoids sometimes testing positive. Assay variability at the cutoff (and antibody specificity) may explain some of our THC-COOH Positive Category discrepant findings. Initially, we found 8 TESTCUP and 11 ONTRAK discrepant results. Six of the discrepant samples tested negative with both on-site test kits, therefore, 13 total sample had results that were inconsistent with the ONLINE findings. These 13 samples were retested with ONLINE and all but one of the samples that tested positive initially were negative in the repeat analysis. Using these repeat data, there was a 94% agreement between ONLINE and TESTCUP and a 99% agreement between ONLINE and ONTRAK.

As stated above, analyte stability may affect test results. After completion of the initial evaluation, all samples were stored frozen while the data were summarized. Due to the variation in THC-COOH Positive Category results just discussed, all samples in this Category were repeated by ONLINE. The repeat analyses were performed approximately six months after the initial testing and the mean donor sample response (normalized to the calibrator response) had decreased 23.7%. A Paired t-test of the data demonstrated that this was a statistically significant decrease between the initial and retest responses ($p = 0.0003$). Analyte stability may be affected by nature of the analyte, quality of the sample, storage conditions, freeze-thaw cycles, time and many other factors and must be considered when reviewing this and similar studies designed to compare drug testing techniques.

A problem with many evaluations of on-site test kits is that the kits are compared to immunoassays that have different antibody specificities and cutoffs concentrations. Ferrara et al., 1994, reported a study that was designed to compare multiple on-site test kits, chromatographic, immunoassay and GC/MS drug test results ($n = 635$) (8). ONTRAK test kits were evaluated by the authors of that study. The authors did not address the differences in antibody cross reactivities and the potential for multiple drugs per drug class in the samples and, consequently, their results were not replicate here as shown by calculating the sensitivity and specificity for TESTCUP and ONTRAK.

Where:

$$\text{Sensitivity} = \text{True Positive} \times 100 / (\text{True Positive} + \text{False Negative})$$

$$\text{Specificity} = \text{True Negative} \times 100 / (\text{True Negative} + \text{False Positive})$$

Ferrara et al., 1994, reported that the sensitivity of ONTRAK for BZE was 74.5, opiates 95.1 and THC-COOH 73.2 (8). However, True positive samples were not identified by a single, common and definitive analytical method in that study. Results from that study are in contrast to those reported by Armbruster et al., 1992, who reported that the sensitivity of the ONTRAK BZE test was 98 and that of the THC-COOH test was 94 (7). The data reported here show that the sensitivity of ONTRAK was 91.7, 100 and 90.1 respectively for BZE, opiates and THC-COOH. TESTCUP calculated sensitivity was 98.0, 100 and 92.6 respectively for BZE, opiates and THC-COOH. These reported sensitivities are also applicable when compared to GC/MS since all positive category samples were confirmed by GC/MS.

Ferrara, et al., 1994, reported that the specificity of ONTRAK for BZE was 95.7, opiates 93.8 and THC-COOH 98.3 (8). These results are also not as good as those reported by Armbruster et al., 1992, who found the specificity of the ONTRAK BZE and THC-COOH tests were 100% (7). The data reported here show that the specificity of ONTRAK was 100 for all 3 drugs. The specificity of TESTCUP was 100, 100 and 99.6 respectively for BZE, opiates and THC-COOH.

The sensitivity and specificity for TESTCUP analysis of QC

samples was 100 for BZE, opiates and THC-COOH respectively. The calculated ONTRAK sensitivities were 97.7, 100 and 100 for BZE, opiates and THC-COOH respectively. The specificity of ONTRAK testing was 100 for the three tested drug classes.

The data presented demonstrate that ONTRAK and TESTCUP are effective urinalysis drug testing techniques. These tests have been successfully used in other laboratories and the data presented here either replicate or show improved accuracy over their findings. Each screening technique has advantages. ONTRAK is portable, the test is easily performed and a single drug class can be tested if indicated. TESTCUP is self contained, tests for the most common drugs of abuse simultaneously and the technician is not required to handle the urine specimen. Therefore, each has a niche in urinalysis drug testing. The reliability of these tests was comparable to those of ONLINE. Both ONTRAK and TESTCUP performed favorably with ONLINE in identifying drug negative and drug positive samples. These data indicate that on-site testing and on-site test kits can be used to supplement laboratory based testing with confidence that the drug test results will be comparable.

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