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CEDIA for Screening Drugs of Abuse in Urine and the Effect of Adulterants

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ABSTRACT: The performance of the Microgenics CEDIA® DAU assays for screening amphetamines, barbiturates, benzodiazepines, cocaine, opiates, phencylidine (PCP), and tetrahydrocannabinol (THC) was evaluated on the Boehringer Mannheim/Hitachi 717 in urine. Limits of detection ranged from 0.6 ng/mL for PCP, to 34.1 ng/mL for benzodiazepines. The average within run and total precision for these assays ranged from 1.3 to 7.3% for controls at cutoff concentrations, and control values at −25% and +25% of cutoffs. The rate separations by CEDIA between the negative and cutoff calibrators for all drugs were greater than corresponding EMIT II (Syva Co.) assays. The relative sensitivity and specificity of CEDIA as compared to EMIT II were 95.6 and 98.8%, respectively, on 13,535 urine samples. All positive samples, and those samples producing discordant results between the assays were confirmed by quantitative gas chromatography/mass spectrometry (GC/MS). Using SAMHSA cutoff limits (and including barbiturates and benzodiazepines at 300 ng/mL), the relative sensitivity and specificity of CEDIA vs. EMIT II were 96.7 and 98.8%, respectively. The overall sensitivity of CEDIA vs. GC/MS was 98.9% with 179 false positives, as compared to 96.2% with 189 false positives for EMIT II vs. GC/MS. The effect of adulterants added to urine to potentially invalidate screening results was also tested. CEDIA produced strong interferences for most drug assays in the presence of glutaraldehyde, detergent, and high concentrations of bleach and Drano. Minimal or selective interferences were seen with golden seal tea lemon juice, Visine, and low concentrations of bleach and Drano. Essentially no interference was observed with bicarbonate, sodium chloride, and vinegar.

KEYWORDS: toxicology, enzyme immunoassay, gas chromatography/mass spectrometry, workplace drug testing, adulteration

Testing for drugs of abuse in urine in the workplace is regulated by the Substance Abuse and Mental Health Services Administration (SAMHSA, formerly the National Institute on Drug Abuse). Guidelines mandate screening by immunoassay followed by confirmation by gas chromatography/mass spectrometry [*1*]. Widely used laboratory-based screening assays include enzyme immunoassay [*2,3*], and fluorescence polarization immunoassay (FPIA) [*4*]. Other bedside (point-of-care) immunoassays are also available but because of cost, they are not widely used in high-volume reference laboratory settings [*5*].

The cloned enzyme donor immunoassay (CEDIA) technique uses genetically-engineered fragments of *E. coli* β-galactosidase [*6*]. The activity of this enzyme requires assembly of the two fragments, termed the enzyme acceptor (EA), and enzyme donor (ED). The ED fragment is conjugated to the ligand and competes with the unlabeled ligand for binding to specific monoclonal antibodies. Low concentration of the analyte facilitates binding of the ED-ligand conjugate to the ligand antibody, blocking assemblage to the EA fragment, resulting in low β-galactosidase activity when the substrate is added. High concentrations of the analyte binding to the ligand analyte antibody facilitates assemblage of the EA to ED resulting in high β-galactosidase activity with substrate addition. CEDIA assays are commercially available for therapeutic drugs and hormones and compare well with other homogeneous nonisotopic immunoassays [*7–8*].

The seven CEDIA urine drugs of abuse assays (amphetamines, barbiturates, benzodiazepines, cocaine, opiates, PCP and THC) were evaluated for within-run and total precision, limit of detection, method comparison of human urine samples against the Syva EMIT II assay, using GC/MS results as the definitive assay, and the effect of various adulterants added to potentially invalidate CEDIA test results.

Materials and Methods

Study Sites

Parts of this study were conducted at four different study sites: Methodist Hospital of Indiana Inc, Indianapolis, IN (K. Sun), Medtox Laboratories, St. Paul, MN (G. Hemphill), MedExpress Laboratories, Memphis, TN (R. Foery), and Hartford Hospital, Hartford, CT (A. Wu). Table 1 lists the drugs and the sites where the evaluations were conducted. The Hitachi 717 (Boehringer Mannheim Corporation, Indianapolis, IN) was used at all sites. CEDIA reagents were from Microgenics Corporation (Concord, CA). EMIT II reagents were from Syva Co. (Palo Alto, CA). Positive (+25% of cutoff) and negative (−25% of cutoff) controls were used in all studies (Microgenics Corp. and Medical Analysis Systems, Camarillo, CA). The cutoff concentrations for the

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CEDIA screening assay used in this study were amphetamines 1000 ng/mL, cocaine 150 and 300 ng/mL, opiates 300 ng/mL, PCP 25 ng/mL, THC 25, 50, and 100 ng/mL, barbiturates 200 and 300 ng/mL, and benzodiazepines 200 and 300 ng/mL. The cutoff concentrations for the GC/MS confirmation assays were amphetamines 500 ng/mL, cocaine 150 ng/mL, opiates 300 ng/mL, PCP 25 ng/mL, THC 15 ng/mL, barbiturates 200 ng/mL, and benzodiazepines 200 ng/mL.

Limit of Detection and Precision

The limit of detection (LOD) was calculated by adding three standard deviations to the mean of twenty replicates of the zero calibrator. An average LOD was computed from values obtained at each evaluation site. For precision, a modified "midi" NCCLS protocol was used [*9*]. Two controls (±25% of cutoff concentration) and the cutoff calibrator were assayed in 6 replicates each day for 10 runs (1 run per day within 14 days).

Method Comparison

Rate separations between the negative calibrator (blank) to the cutoff calibrator were measured in milliabsorbance units change per min (ΔmAU/min). Results were compared against observed values for EMIT II published with each lot.

A total of 13,535 urine samples were assayed by the CEDIA and EMIT II assays on the same day. Samples were obtained from those routinely submitted to and assayed by the laboratories conducting these studies. The sensitivity of CEDIA and EMIT II were calculated using gas chromatography/mass spectrometry as the accepted reference standard. Sensitivity was computed as the number of true positive (TP) results divided by the sum of true positives (TP) plus false negatives (FN) [*10*]. Results were defined as falsely negative if screening results were below the screening cutoff levels on urine samples that contain the target drug, at concentrations above GC/MS cutoff concentrations. The cumulative sensitivity was computed by combining results of the five drug classes tested under SAMHSA guidelines (at SAMHSA cutoff concentrations) plus barbiturates and benzodiazepines.

GC/MS was only performed on the 1012 samples positive by either screening assay. Thus the specificity of CEDIA and EMIT II vs. GC/MS was not determined because screened-negative samples were not further tested. Instead, the number of known false positives were tabulated. All laboratories used the Hewlett Packard Mass Selective Detector (Palo Alto, CA), in the selected ion monitoring mode for confirmation analysis. Standard operating procedures in use at these laboratories were followed.

The relative sensitivity (or % agreement among positive samples) and specificity (% agreement among negative samples) between CEDIA vs. EMIT II was also determined, recognizing that EMIT is not an accepted standard. The relative sensitivity was computed as above. The relative specificity was computed as the number of true negatives (TN) divided by the sum of true negatives (TN) plus false positives (FP). Results were defined as falsely positive if screening results were above the screening cutoff levels on urine samples that contained drug at concentrations below the GC/MS cutoff concentrations.

Adulteration Studies

The ability of various household substances to alter urine drug screening results was examined. Drug-free urine was pooled and spiked with methanolic drug standards to levels at or slightly above cutoff concentrations: 1300 ng/mL for amphetamines, 300 and 400 ng/mL for barbiturates and benzodiazepines, 200 and 400 ng/mL for cocaine, 400 ng/mL for opiates, 40 ng/mL for PCP, and 40, 70, and 130 ng/mL for THC. The adulterants studied were 50 g/L sodium bicarbonate, 10 and 100 mL/L bleach (Chlorox), 10 mL/L dishwashing detergent (A & P brand), 1 and 20 mL/L, Drano, 10 mL/L glutaraldehyde, 10 mL/L golden seal Tea, 330 mL/L lemon juice concentrate, 50 g/L salt, 100 mL/L vinegar, and 330 mL/L Visine (Pfizer, New York, NY).

Interference was defined as **strong** (++) if reaction rates were decreased by more than 100 ΔmAU/min in the presence of the adulterant. Under these conditions, falsely negative results are likely to occur even if the urine is strongly positive. Interference was defined as **weak** (+) if the interferant only moderately decreased the reaction rate (<100 ΔmAU/min). False negative results might occur if the unadulterated urine contains a drug at borderline concentrations. **No** (−) interference was defined as the absence of a significant change in reaction rate after addition of the adulterant.

Results

Table 1 tabulates the limits of detection for each of the drugs studied. For each drug, the results presented are the average of all of the reporting sites. In all cases, the limit of detection was at least 15-fold lower than SAMHSA limits. Table 2 lists the average within-run and total precision for CEDIA for all sites. These values are well within the manufacturer's specified limits. Table 3 shows typical rate separations between the zero and cutoff calibrator for CEDIA vs. EMIT II.

Table 4 summarizes the comparison of CEDIA screen results against EMIT II and GC/MS. For the amphetamines, CEDIA and EMIT II produced no false negative results. There were 35 and 47 false positive results for CEDIA and EMIT II, respectively, when compared to GC/MS. Most of these were due to the presence of sympathomimetic amines such as ephedrine, pseudoephedrine, and phentermine. There were a total of 19 samples that were EMIT II positive and CEDIA negative, and 7 that were EMIT II negative and CEDIA positive. These results indicate that there are significant differences in the specificity of the antibodies used in these assays.

The CEDIA barbiturate assay was evaluated at two cutoff limits. CEDIA results at the 200 ng/mL cutoff limit agreed very well against GC/MS with no false negatives, and 18 false positives. In 17 of these 18, butalbital was present in concentrations ranging from 80 to 180 ng/mL. In the remaining sample, which was also positive by EMIT, another drug of the barbiturate class was found by GC/MS (although not specifically identified). Twelve samples were positive by CEDIA and negative by EMIT II. GC/MS analysis revealed that all of these samples contained phenobarbital at concentrations ranging from 510 to 3100 ng/mL. Two samples were negative by CEDIA and positive by EMIT II. One sample contained butalbital (5 ng/mL) and pentobarbital (10 ng/mL) while the other contained butalbital (74 ng/mL) and secobarbital (53 ng/mL). Similar results were seen using the 300 ng/mL cutoff. In 21 false positive results of CEDIA vs. GC/MS, all but three contained butalbital at concentrations ranging from 101 to 260 ng/mL. There were no false negative results for CEDIA. The EMIT II assay vs. GC/MS had a reduced number of false positive results [*13*], but had many more false negative results [*34*]. Most of these samples had phenobarbital at concentrations exceeding the cutoff of 300 ng/mL.

TABLE 1—*Average limits of detection for CEDIA drugs of abuse assays.*

Drug class (cutoff concentrations)	Testing sites	Average LOD, ng/mL	% of SAMHSA Cutoff
Amphetamine (1000 ng/mL)	c	18.1	0.2
Barbiturates (200, 300 ng/mL)	b,c	5.9, 10.2	NA
Benzodiazepines (200, 300 ng/mL)	b	32.5, 34.1	NA
Cocaine (150, 300 ng/mL)	b	7.3	2.4
Opiates	a,b,c	13.2	4.4
PCP	d	0.6	2.4
THC (25, 50, 100 ng/mL)	b,c,d	1.1, 3.2, 5.2	6.4

a = Methodist Hospital, b = MedTox, c = MedExpress, d = Hartford Hospital.
NA = Not applicable.

TABLE 2—*Within-run and total precision for CEDIA drugs of abuse assays.*

	Within-Run (%CV)			Total (%CV)		
Drug class	−25%	cutoff	+25%	−25%	cutoff	+25%
Amphetamines	7.1	4.6	6.9	5.5	5.1	6.9
Barbiturates-200	3.7	2.9	5.4	4.9	3.8	7.3
Barbiturates-300	3.4	2.3	4.9	4.2	3.0	5.8
Benzo.-200	4.0	2.7	5.5	7.2	5.3	7.6
Benzo.-300	3.6	3.1	3.3	5.1	3.8	4.4
Cocaine-150	2.9	2.2	2.3	3.5	3.3	3.1
Cocaine-300	2.3	1.7	1.6	2.9	2.7	2.1
Opilates	3.1	2.9	3.5	4.7	3.9	4.6
PCP	1.9	1.6	1.8	2.6	2.7	2.7
THC-25	3.1	2.9	2.4	6.0	4.0	3.5
THC-50	2.1	2.6	1.3	3.1	3.4	2.5
THC-100	2.1	2.5	1.6	3.0	3.5	2.5

TABLE 3—*Summary of rate differences (ΔmAU/min) between the zero and cutoff calibrators.*

	CEDIA	EMIT II[a]	
	Observed Separation	Observed Rates	Lot No.
Amphetamines	−113	−45	F5
Barbiturates-200	−132	−83	G1
Barbiturates-300	−188	−141	G1
Benzo-200	−106	−34	G1
Benzo-300	−150	−67	G1
Cocaine-300	−185	−55	G1
Opiates	−165	−41	G1
PCP	−122	−91	F2
THC-25/20[b]	−182	−55	F4
THC-50	−198	−70	F2
THC-100	−178	−60	F5

[a]Lot-specific data supplied with the assay.
[b]25 ng/mL cutoff for CEDIA, 20 ng/mL for EMIT II.

The relative sensitivity of the CEDIA benzodiazepine assay at the 200 ng/mL cutoff compared to EMIT II was 99% with only one discordant result. This sample contained desmethyldiazepam. For relative specificity, CEDIA was positive for 12 samples compared to EMIT II: 6 contained sertraline (a non-tricyclic antidepressant), 3 had embramine (antihistamine), 1 had phenelzine (monoamine oxidase inhibitor), 1 each with oxazepam and α-hydroxy alprazolam near the cutoff limit. Comparing CEDIA to GC/MS, there were no false negative results and two additional false positive results due to desmethyldiazepam and oxazepam at near cutoff concentrations. The use of the 300 ng/mL cutoff reduced the number of false positives down to 11 for CEDIA vs. GC/MS, without any sacrifice of sensitivity.

For the cocaine assay at the 300 ng/mL cutoff limit, there were four false negative CEDIA results on samples that were marginally positive for benzoylecgonine assay by GC/MS, with values ranging from 156 to 217 ng/mL. EMIT II was positive for all of these samples. At the 150 ng/mL cutoff, there was one false positive result by CEDIA (GC/MS value 143 ng/mL) and two false positive results by EMIT II (GC/MS 114 and 143 ng/mL).

The CEDIA and EMIT II opiate assays have significant cross-reactivities against morphine metabolites and other opiates not listed under SAMHSA regulations, such as hydrocodone, hydromorphone, and opiate metabolites. Using GC/MS as the standard, CEDIA produced 107 false positive results compared to 123 for EMIT II. In addition, there were four false negative results for EMIT II, with morphine concentrations of 317, 324, 380, and 745 ng/mL by GC/MS. Each of these were appropriately positive by CEDIA.

Good correlation was observed for the PCP assay, with two false positive results for CEDIA as compared to GC/MS. One sample had a GC/MS concentration for PCP of 15 ng/mL, and the other contained diphenhydramine. The EMIT II assay was also positive for the sample containing 15 ng/mL, and was negative for the sample containing diphenhydramine.

THC was evaluated at three different cutoff limits. The CEDIA limit of 25 ng/mL was compared against EMIT II at 20 ng/mL, and GC/MS at 15 ng/mL for the Δ^9-carboxy-THC metabolite. CEDIA and EMIT II were each falsely negative for 2 samples each, with GC/MS values of 15 and 16 ng/mL. CEDIA and EMIT II were falsely positive on 15 and 26 samples, respectively, with Δ^9-carboxy-THC quantitation ranging from 4 to 14 ng/mL. Increasing the cutoff concentration to 50 ng/mL decreased the number of false positive results (3 for CEDIA, 4 for EMIT II) without affecting the number of false negatives for EMIT II, and increasing the number for CEDIA to 8. Use of the 100 ng/mL cutoff resulted in elimination of false positives for CEDIA and reduced the number for EMIT II to 1, while increasing the false negatives to 15 and 7 for CEDIA and EMIT II, respectively.

The cumulative and relative sensitivity and relative specificity for CEDIA vs. EMIT II is shown in Table 4, along with the sensitivity for CEDIA vs. GC/MS and EMIT II vs. GC/MS. Compared to GC/MS, CEDIA has higher sensitivity than EMIT II, largely because of better detection of barbiturates. CEDIA also has a lower number of total false positives, largely due to better specificity for amphetamines and opiates.

The results of the adulteration study are presented in Table 5. Strong interferences for nearly all CEDIA assays were observed with detergent, glutaraldehyde, and high concentrations of bleach

TABLE 4—*Cumulative and relative sensitivity and relative specificity for drugs of abuse assays.*[a]

Drug class	CEDIA vs. EMIT II		CEDIA vs. GC/MS		EMIT II vs. GC/MS	
	Relative sensitivity	Relative specificity	Sensitivity	No. false positives	Sensitivity	No. false positives
Amphetamines	86.8 (125/144)	99.6 (1857/1864)	100 (97/97)	35	100 (97/97)	47
95% CI[b]	81–92	99–100	—		—	
Barbiturates-200	99.0 (200/202)	99.4 (1878/1890)	100 (194/194)	18	93.8 (182/194)	20
95% CI	98–100	99–100	—		90–97	
Barbiturates-300	100 (157/157)	97.9 (1924/1966)	100 (178/178)	21	80.9 (144/178)	13
95% CI	—	97–99	—		75–87	
Benzo.-200	99.0 (98/99)	98.7 (906/918)	100 (96/96)	14	100 (96/96)	3
95% CI	97–100	98–99	—		—	
Benzo.-300	99.0 (95/95)	98.8 (910/921)	100 (95/95)	11	100 (95/95)	1
95% CI	97–100	98–100	—		—	
Cocaine-150	99.1 (106/107)	100 (892/892)	100 (105/105)	1	100 (105/105)	2
95% CI	97–100	98–100	—		—	
Cocaine-300	96.1 (100/104)	100 (895/895)	96.1 (100/104)	0	100 (104/104)	0
95% CI	92–100	—	92–100		—	
Opiates	98.0 (343/350)	97.3 (2850/2930)	100 (231/231)	107	98.3 (227/231)	123
95% CI	97–99	97–98	—		—	
PCP	100 (50/50)	99.9 (948/949)	100 (49/49)	2	100 (49/49)	1
95% CI	—	—	—		—	
THC-25/20[c]	98.8 (318/322)	99.9 (2175/2178)	99.3 (306/308)	15	99.4 (306/308)	26
95% CI	98–100	—	98–100		99–100	
THC-50	96.9 (280/289)	99.9 (2744/2745)	97.2 (278/286)	3	99.7 (285/286)	4
95% CI	95–99	—	95–99		99–100	
THC-100	93.7 (236/252)	99.8 (2850/2587)	94.9 (243/258)	0	97.3 (251/258)	1
95% CI	91–97	—	92–98		95–99	
Overall[d]	96.7 (1150/1180)	98.8 (12,128/12,270)	98.9 (1028/1040)	179	96.2 (1001/1040)	189
95% CI	96–98	98.6–99.0	98–100		95–97	

[a]Numbers of samples shown in parenthesis.
[b]95% confidence interval.
[c]25 ng/mL cutoff for CEDIA, 20 ng/mL for EMIT II.
[d]SAMHSA screening cutoff limits (ng/mL): amph 1000, cocaine 300, opiates 300, PCP 25, and THC 50, plus barb 300, and benzo 300.

and Drano. Selective interferences were observed for golden seal Tea for amphetamines and THC, and Visine for THC. Weak interferences were seen with lemon juice, vinegar, Visine, and low concentrations of bleach and Drano. No interferences were observed with bicarbonate or salt.

Discussion

For certain drugs of abuse assays in urine, the results of this study show that the performance of CEDIA is enhanced when compared to EMIT II. The CEDIA amphetamine assay is more specific than EMIT II, with fewer false positive results with respect to methamphetamine and amphetamine. The CEDIA assay, e.g., has low cross-reactivity towards phentermine. For barbiturates, CEDIA has greater sensitivity towards phenobarbital than the EMIT II assay. Both CEDIA and EMIT II are highly sensitive towards butalbital, with positive results occurring at levels below GC/MS cutoff concentrations. For benzodiazepines, CEDIA does cross-react with sertraline.

As observed with other screening immunoassays, CEDIA for cocaine is very specific, with no demonstrated cross-reactivities towards any drugs besides cocaine metabolites. For the opiates, CEDIA is more sensitive and specific than EMIT II for the targeted drugs, codeine and morphine. Both assays have significant cross-reactivities towards other opiate drugs. In the case of PCP, CEDIA does have some cross-reactivity towards diphenhydramine metabolites. The parent compound cross reacts at 10 μg/mL. For THC, both CEDIA and EMIT II are more sensitive at low cutoff concentrations and more specific at high cutoff concentrations, relative to GC/MS. A screening cutoff of 100 ng/mL is inappropriately high when using a GC/MS cutoff of 15 ng/mL. Hence, SAMHSA has lowered screening cutoff concentrations for THC to 50 ng/mL [*11*], and produces a higher incidence of detection.

There have been numerous studies demonstrating the effect of specific adulterants for drugs of abuse assays [*12–16*]. For example, salt, bleach, and Drano interferes with most EMIT II assays [*12*]. Glutaraldehyde interferes with all EMIT II and RIA assays [*13*]. This adulterant was commercially available as "UrinAid." Visine interferes with screening assays for THC through sequestration of the drug to micelle bodies [*14*]. In the case of hypochlorite, because GC/MS concentrations for some drugs has also been shown to decrease, Baiker et al. have suggested that the drug is chemically altered [*15*]. A summary of adulteration studies has been presented

TABLE 5—*Summary of adulteration studies.*[a]

	Amph	Barb	Benz	Coc	Opi	PCP	THC
Bicarbonate	−	−	−	−	−	−	−
Bleach (1%)	−	+	−	+	−	−	+
Bleach (10%)	++	++	++	++	++	++	++
Detergent	++	++	−	++	++	++	++
Drano (0.1%)	−	−	−	−	−	−	++
Drano (20%)	++	++	++	++	++	++	++
Glutaraldehyde	−	++	+	++	−	++	++
Golden seal tea	++	−	−	−	−	−	++
Lemon juice	+	+	+	+	+	−	+
Salt	−	−	−	−	−	−	−
Vinegar	−	−	−	+	−	−	+
Visine	−	+	+	+	−	−	++

[a]++ strong interference (ΔmAU/min ≥100); + weak interference (ΔmAU/min <100); − no interference (no change in ΔmAU/min).

by O'Connor et al. [16]. Although there are variations in the degree and type of interferences in specific cases, adulteration is a universal problem for all drugs-of-abuse screening assays. The sensitivity of CEDIA towards adulteration appears to be equivalent to EMIT II, although the CEDIA is not interfered with by the presence of 5% salt. Detection of adulterated urine, such as through visual observations and assays for creatinine, specific gravity, and pH, remains an important part of the forensic urine drug testing process. It is also important to monitor reaction rates, as many adulterants produce values that are significantly below those of blank urine.

The Microgenics CEDIA DAU assays offer an alternative to EMIT for drugs of abuse testing, particularly for laboratories using Hitachi analyzers. Reagents are reconstituted without measuring, into bottles that can be placed directly onto these analyzers. Applications for other general chemistry analyzers are currently being developed. Once reconstituted, the reagents are stable for 60 days when stored at 2–8°C. In addition, expected rate differences between the zero and cutoff calibrators are greater for CEDIA than EMIT II (Table 3). This should provide for better discrimination between blank urine and urines containing drugs at cutoff concentrations.

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