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SCREENING FOR HUMAN DRUGS OF ABUSE BY PARTICLE CONCENTRATION FLUORESCENCE IMMUNOASSAY (PCFIA)

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SUMMARY

We have evaluated a panel of Particle Concentration Fluorescence Immunoassay (PCFIA) tests for use in drugs of abuse screening in humans. The tests evaluated include assays for opiates, cocaine, tetrahydrocannabinol, amphetamines, barbiturates and phencyclidine. These tests are exceptionally sensitive and rapid, since PCFIA readers are highly automated.

The I-50 values for the major drugs of abuse in each assay were determined. The cocaine assay was half-maximally inhibited by about 50 ng/ml benzoylecgonine and was much more sensitive to parent cocaine. The amphetamine assay was half-maximally inhibited by 10 ng/ml of d,l-amphetamine, the phencyclidine assay by 1 ng/ml of phencyclidine, the tetrahydrocannabinol assay by 350 pg/ml of 11-nor-delta-8-tetrahydrocannabinol-9-carboxylic acid, the opiate assay by less than 1 ng/ml of morphine and the barbiturate assay by less than 1 ng/ml of secobarbital. The sensitivity of the assays was such that test urines were diluted ten fold prior to testing.

No false positives were observed in a series of 50 control urines and very few false negatives were observed. On cross-reactivity studies the specificity and sensitivity of our panel of PCFIA tests compared favorably with that of the Abbott TDx^{R} system. In our hands the PCFIA system was more sensitive than the TDx and correlated better with the gas chromatograph/mass spectroscopy status of some of these samples. Beyond this, the potential for automation and sample throughput with the Baxter/Pandex Screen Machine and PCFIA technology is currently unmatched by any immunoassay technology.

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INTRODUCTION

The most successful technique for control of drug abuse, misuse, or recreational use is routine drug screening. Such programs are now widely used by many organizations including the armed forces (Irving, 1988; Peat, 1988). They have been credited with substantially reducing drug abuse in populations subjected to testing and are very cost effective.

The success of these programs has, in turn, created a need for rapid, sensitive and inexpensive screening methods for these drugs (Hoyt et al, 1987). The methods need to be sensitive because many abused drugs are of high potency. The methods must be swift, since the routine screening of large numbers of samples is required for this technology to be effective. Ideally, the method should be capable of automation.

Systems based on immunoassay meet most of the requirements outlined above (Sunshine, 1988). For many high potency drugs, immunoassay is the only method that is sensitive enough for drug detection. The immunoassay methods used in drug detection may use radioactivity or, more recently, fluorescence immunoassay or enzyme multiplied immunoassay in their detection mode. Systems which do not require the use of radioactivity have a substantial advantage in that the handling of reagents is simplified and the level of training required is reduced. For these reasons it is highly desirable that the screening method of choice not require the use of radioactive materials.

Recently we have developed a panel of drug tests for use in racing horses based on Particle Concentration Fluorescence Immunoassay (PCFIA) technology (McDonald et al., 1987; Yang et al., 1987; Woods et al., 1988). In this technology using reagents developed by International Diagnostics Systems (IDS), the test sample is allowed to react with an antibody to the

drug and a drug-fluorophore complex in a microtiter well. After an equilibration time of five to ten minutes, second antibody-coated particles are added to the system and the complex concentrated by vacuum at the bottom of the well. The particles are then washed to remove unbound fluorescent material and the fluorescence response is determined. The intensity of the fluoroscence is inversely related to the amount of free drug in the sample. Using this technology a panel of drug tests in racing horses has been developed and used with great success in horse racing.

Because of the proven effectiveness of this technology in equine drug testing, we have adapted it for use in human drugs of abuse screening. We now report on the use of PCFIA in human drug screening with an emphasis on the sensitivity, specificity, speed, and simplicity of this technology. For comparative purposes we elected to compare this system with a well established drug screening technology, the Abbott TDx^R^ fluorescence polarization immunoassay system.

MATERIALS AND METHODS

Particle Concentration Fluorescence Immunoassay (PCFIA)

The instrument used in the PCFIA tests was the Pandex™ Fluorescence Concentration Analyzer (FCA) or Screen Machine (Baxter/Pandex, Mundelein, IL) automated system as previously described (Jolley *et al.*, 1984; McDonald *et al.*, 1987).

The basic functional unit in the Pandex PCFIA is a 96 well plate with a filter base in each plate. To each well is added 20 µl of drug-B-phycoerythrin (drug-BPE), 20 µl of anti-drug antibody and 20 µl of blank, standard, or test sample (Jolley *et al.*, 1984). The system is allowed to equilibrate for about 10 minutes when a second antibody system is added. The second antibody consists of goat anti-rabbit antibody bound to

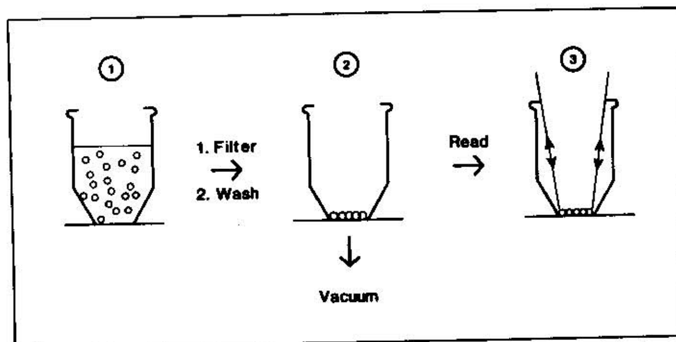

Figure 1 Principle of Particle Concentration Fluoroimmunoassay

Drug in sample is allowed to displace drug-B-phycoerythrin from the drug specific antibody (1). Second antibody coated latex particles are added to the system and the particles drawn down by application of a vacuum (2). The fluorescence of the resulting layer of particles is then measured and the loss of fluorescence estimated (3). (Adapted from Jolley *et al.*, 1984).

latex beads. The system is allowed to react for another 10 minutes and then the fluid is drawn out of the microtiter wells through the filter membrane. The reaction system is then washed with 100 µl of phosphate buffer to resuspend the particles, and the system again drawn down with the vacuum. The filtration step has the effect of concentrating the latex beads 1000-fold, thereby increasing the sensitivity of the method (Figure 1). After the wash step, the fluorescence of the particles at 545 and 575 nm is measured. The mean response from control urines is usually about 25,000-30,000 arbitrary fluorescence units/well. The fluorescent intensity is inversely related to the concentration of free drug or metabolite in the test urine.

Abbott TDx

All cross-reactivity and comparison studies were performed using the Abbott TDx fluorescence polarization immunoassay system (Abbott Laboratories, North Chicago, IL) as previously described (Jolley, 1981; Jolley *et al.*, 1981; Poklis, 1987). The TDx reagents (drug antiserum,

drug-fluorescein complex, assay buffer) were obtained from Abbott Laboratories. An Abbott TDx Analyzer was used to perform the assay.

Drugs

All drugs and metabolites were supplied by the Illinois Racing Board Laboratory and the National Institute on Drug Abuse (Rockville, Maryland).

Urine Samples

Gas chromatography/mass spectroscopy (GC/MS)-confirmed drug-free human urine samples (n = 50) were assayed by PCFIA and TDx for opiates, barbiturates, tetrahydrocannabinol (THC), cocaine, phencyclidine, amphetamine, and their metabolites. Also, for each drug, another group of GC/MS-analyzed human urine samples (n = 54 to n = 86), some of which were confirmed positive containing the drug or its metabolites, were tested by PCFIA and TDx. The human urine samples and the GC/MS analyses were provided by clinical laboratories in Miami, FL; Denver, CO; Chicago, IL and the Illinois Racing Board Laboratory.

These assays were originally developed for trace residue analysis in racing samples, hence the sensitivity is far greater than that needed for routine drug analysis in humans. To compensate for this, all urine samples were diluted 1:10 in buffer prior to adding an aliquot to the reaction well. In the assay sample fluorescent values were compared with those of known or threshold concentration of the drug or metabolite.

Cross-Reactivity Study

Cross-reactivity studies were conducted by spiking known negative urine to a final concentration of 100 µg/ml with the listed drug. These urines were then run in the appropriate test and the results reported as a screening positive or negative.

Assignment of "Positive" Status

Assignment of "positive" status was based on comparison of the instrument reading with the reading yielded by "high" and "low" controls as indicated in Table 1. If the reading on a particular sample exceeded the reading from the low control sample the sample was assigned positive status.

Table 1

Drug	Low Control	High Control
Opiates	250 ng/ml	800 ng/ml
Barbiturates	600 ng/ml	1 µg/ml
THC	35 ng/ml	120 ng/ml
Cocaine	500 ng/ml	3 µg/ml
PCP	35 ng/ml	250 ng/ml
Amphetamines	150 ng/ml	1.5 µg/ml

RESULTS

Cocaine

Figure 2 shows the displacment of cocaine-BPE from the anti-cocaine antibody by benzoylecgonine, a metabolite of cocaine. In the absence of added benzylocgonine the fluorescence counts in the assay system averaged about 27,000 counts. Addition of 1 ng/ml of benzoylecgonine produced about a ten percent inhibition of fluorescence counts in the assay system, with increasing concentrations of benzoylecgonine increasing the inhibition in a log-linear manner. Half-maximal inhibition was observed at about 50 ng/ml of benzoylecgonine. In other experiments half maximal inhibition was obtained with much lower concentrations of parent cocaine.

In a series of cross-reactivity experiments, our PCFIA system reacted strongly with cocaine, ecgonine methyl ester and also with ecgonine itself. This broad cross-reactivity suggested to us that our assay system would readily detect both parent cocaine or its metabolites in forensic samples.

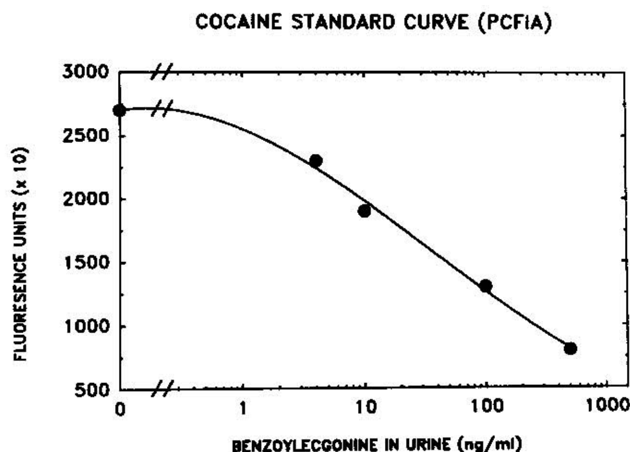

Figure 2. The standard curve for cocaine inhibition of BPE fluorescence in human urine was constructed. The solid circles (•-•) show inhibition of fluoresence in the PCFIA assay for cocaine after the addition of the indicated concentration of benzoylecgonine to the system.

If this is true our assay should give rise to a very low false negative rate for cocaine or its metabolites in human drug screening. We therefore obtained 86 human urine samples, 28 of which had been confirmed positive for cocaine or its metabolites by mass spectral analysis and evaluted the ability of our kit to detect the presence of cocaine or its metabolites in these samples. As a positive control we ran our tests in parallel with the Abbott TDx system, to allow direct comparison of the performance of our kits with an established assay system.

The results of this comparison for cocaine are shown in Tables 2a and 2b. No false positives were observed in approximately 50 drug free human urine samples. On the other hand, when a series of 86 human urine samples of which 28 had been confirmed positive for cocaine or its metabolites were

examined, the PCFIA technology identified 27 of these as containing cocaine or its metabolites, while the TDx system flagged 23 and refused one sample. While the reason for this discrepancy is not clear it may be that the broader specificity of the PCFIA antibodies gives it a lower false negative rate than the Abbott TDx, at least in this evaluation.

We also evaluated the cocaine antibody for cross-reactivity with approximately 50 medications as shown in Table 8. The IDS PCFIA reagents cross-reacted with both cocaine and phenylethylamine. The TDx reagents did not cross-react with any of the 50 medications listed including parent cocaine indicating the specificity of the TDx antibody for benzoylecgonine or other metabolites.

Table 2a.

Analysis of 50 Cocaine Free Urine Samples for False Positives

Analytical Method	Known Negatives	Screen Negatives	False Positives
PCFIA	50	50	0
TDx	50	50	0
GC/MS	50	50	0

Table 2b.

Analysis of Population of Cocaine Positive Samples for Apparent False Negatives

Analytical Method	Total	Positive	Negative	False Positives	False Negatives	Other
GC/MS*	86	28	58	0	0	0
PCFIA	86	27	59	0	1	0
TDx	86	23	63	0	4	1**

*Urines positive for cocaine or its metabolites by GC/MS were obtained from Clinical Laboratories in Miami, Denver, Chicago and the Illinois Racing Board Laboratory. The definition of a screening test positive was the identification of a concentration $\geq$ 500 ng/ml of apparent benzoylecgonine in urine.
**1 urine was refused by the machine.

Amphetamine

The ability of amphetamine to displace amphetamine-BPE from our anti-amphetamine antibody is presented in Figure 3. Concentrations of amphetamine as low as 1 ng/ml were readily able to displace amphetamine-BPE from its antibody binding sites, and half maximal inhibition occured at about 10 ng/ml.

We also elected to evaluate our amphetamine PCFIA assay in parallel with the TDx assay for amphetamine on human urine samples known to contain amphetamine or its metabolites and samples known to contain no amphetamine (Table 3a, b). Of 50 known negative samples each was correctly assigned negative status by both PCFIA and TDx screening. When 54 samples (15 known to be positive for amphetamines via GC/MS) were analyzed by both PCFIA and TDx, no false negatives were observed. One sample, however was refused by the TDx and one sample not known to be positive for an amphetamine like substance yielded a reaction for amphetamine in the PCFIA system.

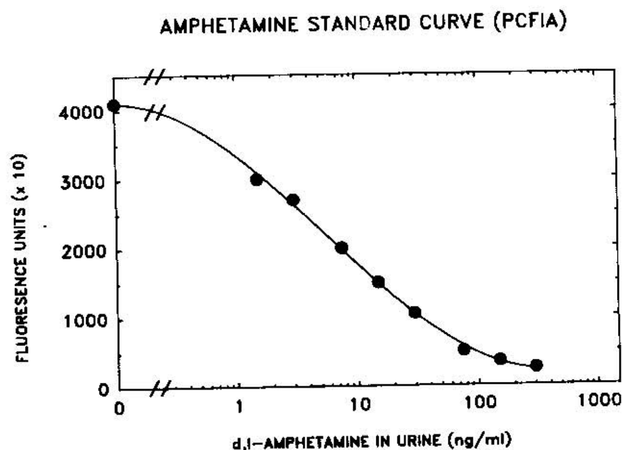

Figure 3. The standard curve for amphetamine inhibition of BPE fluorescence in human urine was constructed. The solid circles (●-●) show inhibition of fluoresence in the PCFIA assay for amphetamine after the addition of the indicated concentration of d,l-amphetamine to the system.

Table 3a

Analysis of 50 Amphetamine Free Urine Samples for False Positives

Analytical Method	Known Negatives	Screen Negatives	False Positives
GC/MS	50	50	0
TDx	50	50	0
PCFIA	50	50	0

Table 3b

Analysis of Population of Amphetamine Positive Samples
for Apparent False Negatives

Analytical Method	Total	Positive	Negative	False Positives	False Negatives	Other
GC/MS	54	15	39	0	0	0
PCFIA	54	16*	38	1*	0	0
TDx	54	14	39	0	0	1*

*A TDx/PCFIA Positive was defined as a concentration greater than or equal to the minimum allowable threshold, 150 ng/ml of d,l-amphetamine.
**TDx refused one sample, and the PCFIA called a sample positive that was not known to be positive by GC/MS, which sample the TDx called negative.

We also evaluated the ability of our amphetamine assay system to cross-react with a panel of about 50 medications as outlined in Table VII. Both the PCFIA and Abbott TDx system reacted well with D-amphetamine, methamphetamine, and β-phenylethylamine. Both systems gave partial reactions with tyramine and phentermine. The TDx system partially reacted with fenfluramine and labetalol which were not detected by the PCFIA system. On the other hand the PCFIA system recognized phendimetrazine and gave an equivocal reaction with a methadone metabolite.

Phencyclidine

The ability of phencyclidine to displace phencyclidine-BPE from the anti-phencyclidine antibody is presented in Figure 4. The system was very sensitive to added phencyclidine with half-maximal inhibition occurring at about 1 ng/ml. When the ability of this test to detect phencyclidine was

compared to the TDx system, both systems detected 20 known phencyclidine containing samples with no false positives and no false negatives (Tables 4a, b).

We also evaluated the cross-reactivity of both the PCFIA and the Abbott TDx system with about fifty therapeutic and illegal medications. Both systems showed some cross-reactivity with levallorphan and levorphanol, both opiate like structures, but no other evidence of cross-reactivity was observed.

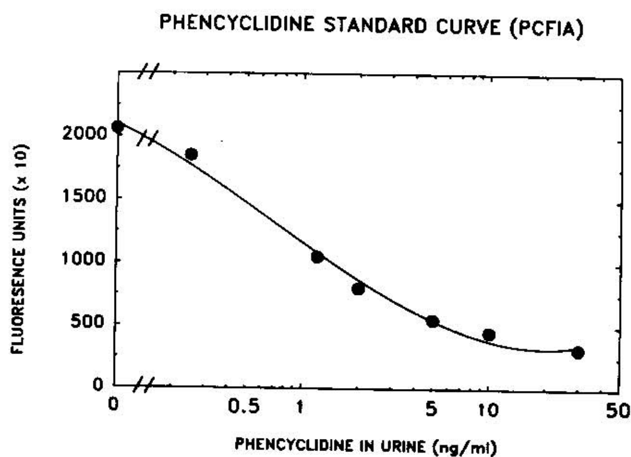

Figure 4. A standard curve for phencyclidine inhibition of BPE fluoresence in human urine was constructed. The solid circles (•-•) show inhibition of fluoresence in the PCFIA assay for phencyclidine after the addition of the indicated concentration of phencyclidine to the system.

Table 4a

Analysis of 50 Phencyclidine Free Urine Samples for False Positives

Analytical Method	Known Negatives	Test Negatives	False Positives
GC/MS	50	50	0
TDx	50	50	0
PCFIA	50	50	0

Table 4b

Analysis of Population of Phencyclidine Positive Samples
for Apparent False Negatives

Analytical Method	Total	Positive	Negative	False Positives	False Negatives	Other
GC/MS	54	20	34	0	0	0
PCFIA	54	20	34	0	0	0
TDx	54	20	34	0	0	0

A PCFIA/TDx positive was defined as a concentration greater than or equal to 35 ng/ml of phencyclidine.

Tetrahydrocannabinol (THC)

The ability of 11-nor-delta-8-tetrahydrocannabinol-9-carboxylic acid to displace our THC-BPE conjugate from the anti-THC antibody is shown in Figure 5. The test is extremely sensitive to added THC, with substantial displacement starting at 250 pg/ml and virtually complete displacement being obtained at levels of less than 1 ng/ml. As with other assays reported in this paper, this extreme sensitivity allows for substantial dilution of urine samples prior to analysis.

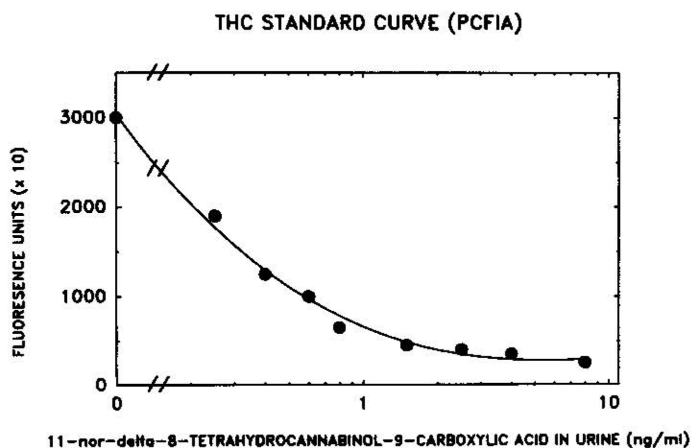

Figure 5. The standard curve for tetrahydrocannabinol (THC) inhibition of BPE fluoresence in human urine was constructed. The solid circles (●-●) show inhibition of fluoresence in the PCFIA assay for THC after the addition of the indicated concentration of 11-nor-Δ-8-tetrahydrocannabinol-9-carboxylic acid to the system.

We further analyzed 85 samples by both PCFIA and TDx to compare the sensitivity and efficacy of these screening systems. As shown in Table 5a, when 50 known negative samples were assayed by both the PCFIA and TDx methods, no false positives were observed. Similarly when a series of 84 samples containing 34 known positives were analysed by both systems the PCFIA system correctly identified the 34 known positives (Table 5b). On the other hand the TDx correctly identified all the positives that the machine would accept, but the TDx refused to accept 4 known positive samples.

We also evaluated the cross-reactivity of our PCFIA system for THC with about fifty other therapeutic and abused medications. In our hands the TDx system appeared to cross-react with isoxuprine whereas the PCFIA system did not recognize isoxuprine. No other forensically significant cross-reactivities were observed in any of our THC cross-reactivity studies.

Table 5a

Analysis of 50 Tetrahydrocannabinol Free Urine Samples for False Positives

Analytical Method	Known Negatives	Test Negatives	False Positives
GC/MS	50	50	0
TDx	50	46*	0
PCFIA	50	50	0

*4 samples were refused by Abbott TDx.

Table 5b

Analysis of Population of Tetrahydrocannabinol Positive Samples for Apparent False Negatives

Analytical Method	Total	Positive	Negative	False Positives	False Negatives	Other
GC/MS	85	34	51	0	0	0
PCFIA	85	34	51	0	0	0
TDx	85	30	51	0	0	4*

A PCFIA/TDx Positive was defined as a concentration greater than or equal to 35 ng/ml 11-nor-delta-8-THC-9-carboxylic acid.
*4 samples were refused by Abbott TDx.

Opiates

The ability of morphine to displace our morphine-BPE conjugate from our anti-morphine antibody is shown in Figure 6. The addition of 200 pg/ml of morphine to the system produced a small displacement of the morphine-BPE conjugate from the system. Half maximal inhibition was observed at about 1 ng/ml, and increasing concentrations of morphine produced virtually complete inhibition of fluorescence. Other studies with this same antibody in the PCFIA kit and in the ELISA format showed that the antibody cross-reacted with codeine, hydromorphine, levorphanol, morphine-3-β-glucuronide, oxycodone, oxymorphone, thebaine, nalorphine, diacetyl-morphine, dihydrocodenin and ethylmorphine (McDonald et al., 1988).

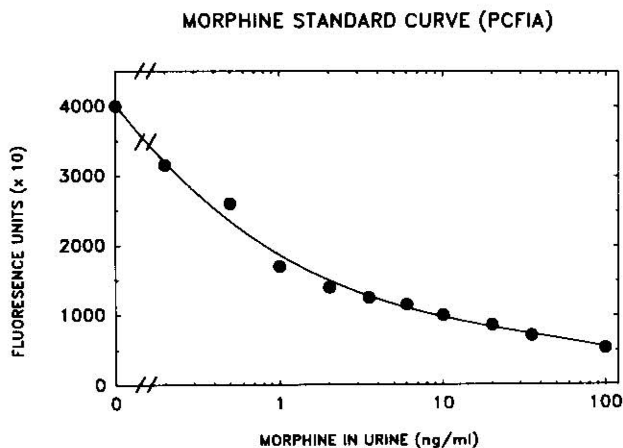

Figure 6. The standard curve for morphine inhibition of BPE fluoresence in human urine was constructed. The solid circles (●-●) show inhibition of fluoresence in the PCFIA assay for morphine after the addition of the indicated concentration of morphine to the system.

When we compared the PCFIA and the TDx tests on 50 known negative samples both assays produced no false positives (Table 6a). When we analysed a population of 78 samples containing 56 known positives by

screening with both the PCFIA and TDx methods, the PCFIA system correctly identified all the known positive samples and the TDx system also correctly identified all but three of the known positive samples (Table 6b). Of the samples that the TDx did not identify two were refused by the machine and one was assigned weak positive status.

Table 6a

Analysis of 50 Opiate Free Urine Samples for False Positives

Analytical Method	Known Negatives	Test Negatives	False Positives
GC/MS	50	50	0
TDx	50	50	0
PCFIA	50	50	0

Table 6b

Analysis of Population of Known Opiate Positive Samples
for Apparent False Negatives

Analytical Method	Total	Positive	Negative	False Positives	False Negatives	Other
GC/MS	78	56	22	0	0	0
PCFIA	78	56	22	0	0	0
TDx	78	53	22	0	0	3*

A PCFIA/TDx positive was defined as a concentration greater than or equal to 250 ng/ml of morphine.
*2 samples were refused by the Abbott TDx while one was classified as a weak positive.

We also examined the cross-reactivity of our PCFIA system and the Abbott TDx system opiates with about fifty therapeutic medications or drugs of abuse. As shown in Table 8, both systems recognized codeine, thebaine and oxycodone. The PCFIA system gave an equivocal reaction with diphenylhydantoin, delta-THC and pramoxine. On the other hand the TDx system appeared to react with phendimetrazine, cross-reacted with nalorphine and gave an equivocal reaction with meperidine, as shown in Table 8.

Barbiturates

The ability of secobarbital to displace our barbiturate-BPE conjugate from our anti-barbiturate antibody is presented in Figure 7. As with the other tests, the system is very sensitive to the presence of added barbiturates, with half maximal inhibition of fluoresence occurring after addition of about 1 ng/ml of secobarbital. The antibody cross-reacts well with other barbiturates, including allobarbital, alphenol, amobarbital, aprobarbital, barbital, butabarbital, pentobarbital, phenobital, talbutal and secobarbital.

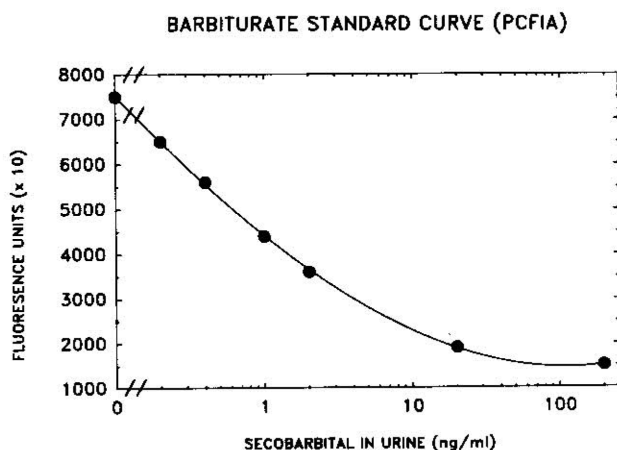

Figure 7. The standard curve for barbiturate inhibition of BPE fluoresence in human urine was constructed. The solid circles (•-•) show inhibition of fluoresence in the PCFIA assay for barbiturate after the addition of the indicated concentration of secobarbital to the system.

When we analyzed 50 known drug free urines for barbiturates by both the TDx and our PCFIA system, no false positives were observed (Table 7a). In addition we analyzed a population of 85 samples, 23 of which were known barbiturate positives. Both systems correctly identified all 23 barbiturate positives (Table 7b), with no sample refusals by the TDx machine.

Table 7a

Analysis of 50 Barbiturate Free Urine Samples for False Positives

Analytical Method	Known Negatives	Test Negatives	False Positives
GC/MS	50	50	0
TDx	50	50	0
PCFIA	50	50	0

Table 7b

Analysis of Population of Known Barbiturate Positive Samples
for Apparent False Negatives

Analytical Method	Total	Positive	Negative	False Positives	False Negatives	Other
GC/MS	85	23	62	0	0	0
PCFIA	85	23	62	0	0	0
TDx	85	23	62	0	0	0

A PCFIA/TDx positive was defined as a concentration greater than or equal to 600 ng/ml of secobarbital.

We also examined the cross-reactivity of both our PCFIA and TDx barbiturate detection systems with about fifty therapeutic medications and drugs of abuse. As shown in Table 8, both systems recognized aprobarbital. The TDx system gave an equivocal reaction with dilantin, while both systems gave an equivocal reaction with mephobarbital. Both systems recognized phenobarbital and pentobarbital, and no other significant cross reactivities were noted.

Table 8

Comparison of Cross-Reactivity of Drug Antibodies in the PCFIA and TDx Systems

Antibody	Amphetamine		Cocaine		THC		Barbiturate		Opiate		Phencyclidine	
System	PCFIA	TDx	PCFIA	TDx	PCFIA	TDx	PCFIA	TDx	PCFIA	TDx	PCFIA	TDx
DRUG												
Allobarbital	-	-	-	-	-	-	-	-	-	-	-	-
Alphaprodine	-	-	-	-	-	-	-	-	-	-	-	-
D-Amphetamine	+	+	-	-	-	-	-	-	-	-	-	-
Aprobarbital	-	-	-	-	-	-	+	+	-	-	-	-
Benzocaine	-	-	-	-	-	-	-	-	-	-	-	-
Chlorpheniramine	-	-	-	-	-	-	-	-	-	-	-	-
Chlorpromazine	-	-	-	-	-	-	-	-	-	-	-	-
Cocaine	-	-	+	-	-	-	-	-	-	-	-	-
Codeine	-	-	-	-	-	-	-	-	+	+	-	-
Diphenoxylate	-	-	-	-	-	-	-	-	-	-	-	-
Diphenylhydantoin	-	-	-	-	-	-	-	+/-	-	-	-	-
Doxepin	-	-	-	-	-	-	-	-	+/-	-	-	-
Ephedrine	-	-	-	-	-	-	-	-	-	-	-	-
Fenfluramine	-	+/-	-	-	-	-	-	-	-	-	-	-
Hexobarbital	-	-	-	-	-	-	-	-	-	-	-	-
Ibuprofen	-	-	-	-	-	-	-	-	-	-	-	-
Isoxuprine	-	-	-	-	-	+	-	-	-	-	-	-
Ketoprofen	-	-	-	-	-	-	-	-	-	-	-	-
Labetalol	-	+/-	-	-	-	-	-	-	-	-	-	-
Levallorphan	-	-	-	-	-	-	-	-	-	+	+	+/-
Levorphanol	-	-	-	-	-	-	-	-	+	+	+/-	+/-
Lidocaine	-	-	-	-	-	-	-	-	-	-	-	-
Meperidine	-	-	-	-	-	-	-	-	-	+/-	-	-
Mephobarbital	-	-	-	-	-	-	+/-	+/-	-	-	-	-
Methamphetamine	+	+	-	-	-	-	-	-	-	-	-	-
Metharbital	-	-	-	-	-	-	-	-	-	-	-	-
Methadone metabolite	+/-	-	-	-	-	-	-	-	-	-	-	-

Antibody System	Amphetamine		Cocaine		THC		Barbiturate		Opiate		Phencyclidine	
	PCFIA	TDx	PCFIA	TDx	PCFIA	TDx	PCFIA	TDx	PCFIA	TDx	PCFIA	TDx
DRUG												
Nalorphine	-	-	-	-	-	-	-	-	-	+	-	-
Naloxone	-	-	-	-	-	-	-	-	+/-	+/-	-	-
Naproxen	-	-	-	-	-	-	-	-	-	-	-	-
Nortriptyline	-	-	-	-	-	-	-	-	-	-	-	-
Oxycodone	-	-	-	-	-	-	-	-	+	+	-	-
Pentazocine	-	-	-	-	-	-	-	-	-	-	-	-
Pentobarbital	-	-	-	-	-	-	+	+	-	-	-	-
Phencyclidine	-	-	-	-	-	-	-	-	-	+/-	+	+
Phendimetrazine	+	-	-	-	-	-	-	-	-	-	-	-
Phenobarbital	-	-	-	-	-	-	+	+	-	-	-	-
Phentermine	+/-	+/-	-	-	-	-	-	-	-	-	-	-
Phenylephrine	-	-	-	-	-	-	-	-	-	-	-	-
β-Phenylethylamine	+	+	+	-	-	-	-	-	-	-	-	-
Phenylpropanolamine	-	-	-	-	-	-	-	-	-	-	-	-
Pramoxine	-	-	-	-	-	-	-	-	+/-	-	-	-
Protriptyline	-	-	-	-	-	-	-	-	-	-	-	-
THC, delta-1	-	-	-	-	+	+	-	-	+/-	-	-	-
THC, delta-6	-	-	-	-	+	+	-	-	-	-	-	-
Thebaine	-	-	-	-	-	-	-	-	+	+	-	-
Thioridazine	-	-	-	-	-	-	-	-	-	-	-	-
Thiothixine	-	-	-	-	-	-	-	-	-	-	-	-
Tyramine	+/-	+/-	-	-	-	-	-	-	-	-	-	-

The table shows the cross-reactivity of the amphetamine, cocaine, THC, barbiturate, morphine (opiate), and phenycyclidine antibodies with 49 pharmacologically active coumpounds in both the PCFIA and TDx systems. Cross-reactivity is indicated by "+", lack of cross-reactivity by "-", and a weak reaction by "+/-".

DISCUSSION

The sensitivity of any drug test is an essential consideration in determining its suitability for use in routine drug screening. If the test is insufficiently sensitive it will not detect small concentrations of drugs or drug metabolites, thus give rise to false negatives. Sensitivity is therefore a prime requisite for an effective screening test. A further advantage of a highly sensitive test is that samples can be diluted before being introduced into the test. This dilution has the effect of reducing the concentration of interfering substances, thereby increasing the accuracy of the test.

The PCFIA tests reported in this paper are highly sensitive to the point that urine samples containing drugs could be diluted ten fold prior to use without any loss of sensitivity. As shown in Table 9, the ratios between the I-50 values for parent drugs and the threshold for "positive" calls on drugs in each of these tests are in each case at least 15 fold. For each of these PCFIA tests a substantial reserve of sensitivity existed which enabled

Table 9

Ratios Between PCFIA I-50 Values and Thresholds for "Positive" Calls

DRUG	I-50, PCFIA	POSITIVE PCFIA/TDx	RATIO THRESHOLD
Cocaine	3 ng/ml	500 ng/ml benzoylecgonine	160
Benzoylecognine	50 ng/ml	500 ng/ml benzoylecgonine	60
Phencyclidine	1 ng/ml	75 ng/ml phencyclidine	75
THC	0.35 ng/ml	35 ng/ml Δ-THC carboxylic acid	
Morphine	<1 ng/ml	250 ng/ml morphine	250
Secobarbital	1 ng/ml	600 ng/ml secobarbital	800
Amphetamine	10 ng/ml	150 ng/ml d,l-amphetamine	15

The second column shows the I-50 values for inhibition of the fluorescence reaction by the indicated drug, the next column shows the threshold for a "positive" call in both the PCFIA and TDx systems and the far right hand column shows the ratio between the I-50 value and the threshold value for a positive call.

us to dilute the urine samples ten fold and still easily detect the presence of drugs of abuse in all samples that were submitted for evaluation.

As well as being sensitive the specificity of any drug screening test is important. Specificity is, however, a relative term. Ideally a test should react with the major metabolite or parent form of a drug of abuse and any congeners of the drug that are likely to be abused. Since abuse patterns are not stable and may vary with different geographic areas, it is not possible to develop absolute guidelines concerning specificity. However, as a first approximation a useful test should only pick up drugs of abuse and not detect over-the-counter medications that are likely to be found in human samples and give rise to false positives.

Analysis of the data presented in this communication shows that our PCFIA tests compare very favorably with the Abbott TDx system with regard to specificity. For example, in our analysis of cocaine positive urines the TDx machine refused to accept a number of samples thus giving rise to a significant deficit in the efficacy of testing for this drug. Beyond this, cross-reactivity studies showed that the TDx antibody did not appear to react well with parent cocaine, giving rise to the possibility of false negatives for samples containing predominantly parent cocaine.

Analysis of the data obtained with amphetamine showed that the PCFIA was very sensitive to amphetamine and produced no false negatives when analyzing 50 known negative urines. When the PCFIA was used in a series of samples containing known amphetamine positives, it called one sample positive that was not known to be positive. This sample must therefore be considered a false positive, the only such reaction in this series of tests.

Our phencyclidine assay was similarly highly sensitive to phencyclidine with an I-50 for parent phencyclidine of less than 1 ng/ml. Overall, both

systems reacted very similarly with phencyclidine, with no false positives or false negatives and broadly similar cross-reactivities.

The THC test was extremely sensitive to added 11-nor-delta-8-THC-9-carboxylic acid, with substantial displacement occurring at about 0.25 ng/ml. Both systems correctly identified all the positives that the machine would accept, but the TDx failed to accept four of our THC samples. In our cross-reactivity studies the TDx appeared to cross-react with isoxuprine, while no significant cross-reactivity was seen within the PCFIA system.

In the opiate assay as little as 1 ng/ml of morphine produced more than half maximal inhibition in our PCFIA system. As benefits an opiate assay, the system showed good cross-reactivity with a wide range of opiates and opiate antagonists. When both systems were evaluated against a population of known positives, the PCFIA system correctly identified all the known positives, while the TDX refused two samples and identified a third as marginally positive.

The barbiturate test was also very sensitive to secobarbital, with half maximal inhibition occurring at about 1 ng/ml. Our antibody showed wide cross-reactivity, and both systems correctly identified all positives presented to them and no samples were refused by the TDx.

A summary of the comparative performance of both instruments is presented in Table 10. The only system to produce an apparent false positive was the PCFIA system, which called one sample positive for an amphetamine or an amphetamine like substance. This sample was a street sample and its medication status cannot be assigned with certainty, therefore this sample must be listed as an apparent false positive. Unfortunately, sufficient sample volume was unavailable to us to perform a complete work up on this sample to determine its medication status.

With regard to false negatives, the PCFIA system produced only one false negative as compared to a number of apparent false negatives in the TDx system. In the cocaine test, the TDx system produced three false negatives and one refusal, for a total of 4 missed positives. In the amphetamine test, the TDx had one refusal, and for THC, another widely abused drug, the TDx showed four refusals. The TDx system also refused 2 opiate test samples, for a total of 10 apparent false negatives, 8 of which were refusals and two of which were false negatives. A summary of these data shows that the PCFIA system produced one apparent false positive and one false negative, while the TDx produced no false positives but had a significant number of refusals (8) and four false negatives.

Table 10

Comparison of False Positives, False Negatives and Refusals for PCFIA and TDx Tests

	False Positives		False Negatives		Refusals	
Drug	PCFIA	TDx	PCFIA	TDx	PCFIA	TDx
Cocaine	0	0	1	4	0	1
Amphetamine	1	0	0	0	0	1
Phencyclidine	0	0	0	0	0	0
THC	0	0	0	4	0	4
Opiates	0	0	0	2	0	2
Barbiturates	0	0	0	0	0	0
TOTALS	1	0	1	10	0	8

The total number of samples evaluated was 441, of which 176 were positive for one or more of the above agents.

With regard to speed and potential of automation, the Baxter/Pandex Screen Machine appears to hold enormous potential. The Screen Machine provides "walk away" automation through computerized control systems that move each up to ten 96 well assay plates through a full process cycle. The microprocessor assesses the assay protocol and schedules each step efficiently. The microprocessor also directs the addition of up to three

reagents, incubations, separations of bound and free fluorescence, washing the solid phase, and reading the plate.

The rapid kinetics of PCFIA technology combined with the automation of the Screen Machine can achieve assay throughput unmatched by any immunoassay technology. As many as 960 samples can be processed, including incubations, in less than two hours. This will be dependent on the type of assay and protocol programmed into the Screen Machine. Beyond this, with the selection of fluorescent tags emitting at different wavelengths, it is possible to perform up to three different assays simultaneously in the same well, leading to substantial increases in sample throughput and savings in reagent costs.

In summary, the studies presented here show that the PCFIA system reported, using reagents developed by International Diagnostic Systems Corp., resulted in a highly sensitive assay system for drugs of abuse. The performance of this system is comparable with, and in some respects superior to, the industry standard in drug screening, the Abbott TDx system. Both systems allow economical, rapid and effective screening of human urine samples for drugs of abuse. Both screening systems generate presumptive evidence of positives only, and all screening suspects must be positively confirmed by an unequivocal confirmatory method such as mass spectrometry.

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