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METHODS OF IDENTIFICATION AND CONFIRMATION OF ABUSIVE DRUGS IN HUMAN URINE

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SUMMARY

A thin-layer chromatography (TLC) procedure is described to be used as the initial drug detection method for urine surveillance in a drug abuse treatment program. While the TLC method is sufficiently sensitive, it is prone to false-positive results. For this reason, two other drug detection methods (gas–liquid chromatography and radioimmunoassay) have been incorporated to confirm positive results obtained with TLC. The combined methodologies result in a urine surveillance procedure that is versatile, sensitive and highly reliable.

INTRODUCTION

The analysis of urine for drugs of abuse is currently the most important method of determining the incidence of drug abuse. Urine surveillance has become essential in monitoring the progress of drug abusers during detoxification and rehabilitation. The need for reliable methods of identifying drugs in urine has resulted in many drug-detection methodologies and several excellent reviews of these methods have been published[1–8]. The method used for urine surveillance must be fast, sensitive, reliable, and relatively inexpensive. In addition, it must be able to detect a large variety of drugs. These requirements have been most adequately met by extraction of the drugs from urine and subsequent separation and identification by thin-layer chromatography (TLC). At present, no other method can detect the wide variety of drugs in the same time and at comparable low cost.

The sensitivity of present TLC procedures ranges from 0.5–1.0 μg/ml of urine for the more commonly abused drugs, and we have found that TLC will detect most drugs for at least 36 h after a therapeutic dose. For urine surveillance where biweekly analysis of patients' urine is performed this sensitivity is adequate. The TLC method, however, has perhaps the highest incidence of false-positive results due to interference by other drugs and non-specific factors. The incidence of false-positive

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results necessitates the use of confirmatory tests in conjunction with TLC. Such confirmatory procedures should not suffer from the same type of interference found with TLC and should be inherently more sensitive to confirm questionable positive results. The present work describes a practical method for urine surveillance, utilizing TLC as an initial screening method followed by two confirmatory procedures, gas–liquid chromatography (GLC) and radioimmunoassay (RIA).

METHODS AND MATERIALS

Chemicals

All drugs used as standards were obtained in pure powdered form from commercial sources. The TLC medium used was Gelman ITLC, type SG (Gelman Instrument, Ann Arbor, Mich., U.S.A.). Amberlite XAD-2® was purchased from Rohm & Haas (Philadelphia, Pa., U.S.A.) and prior to use was washed with three volumes of methanol and ten volumes of distilled water. Abuscreen® radioimmunoassay kits for morphine, barbiturates and amphetamines were obtained from Roche Diagnostics (Nutley, N.J., U.S.A.). Fluoram® was purchased from Scientific Products (McGraw Park, Ill., U.S.A.).

TLC extraction procedure

Drugs were extracted from patient urine using a modification of the method of Fujimoto and Wang[9]. A column was fabricated from a 0.004-in., 12.5-cm × 20-cm polyethylene bag by heat-sealing a wedge-shaped channel from the lower corner to a point midway across the top of the bag[13,20]. The bottom corner of the column was pierced with a 15-gauge syringe needle (Fig. 1). This hole (1 mm diameter) served as

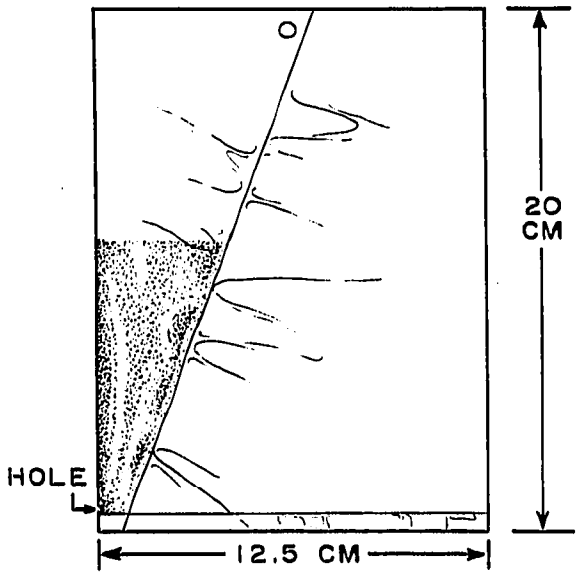

Fig. 1. Disposable plastic bag column filled with Amberlite XAD-2 resin. If eluting solvents with a density greater than that of water are used, a glass wool plug must be placed on top of the Amberlite XAD-2 to prevent disturbance of the resin column.

the outlet of the column. The column was suspended by a hook from a horizontal rod and filled to a height of 12 cm (about 4 g) with an aqueous slurry of the washed Amberlite XAD-2. Excess water was allowed to drain out of the resin, and urine (35 ml) was poured in the top of the bag and allowed to pass through the resin. The bag was compressed slightly to remove as much urine as possible. Drugs were eluted from the resin with 20 ml of methanol. The colored portion of the eluate was collected and 15 ml of this eluate were concentrated by evaporation on a Buchler Evapo-Mix® at 45°. Two-hundred microliters of methanol were added to the viscous residue, and 20 μl of the residue were spotted on the chromatogram.

TLC solvent systems

The concentrated extract from each urine sample was spotted on three different sheets of Gelman ITLC, Type SG. Nine patient samples and one standard were spotted on each chromatogram. Each of the three chromatograms was developed in a different solvent system: (A) benzene–hexane–diethylamine (25:10:1), (B) chloroform–ammonium hydroxide (50:0.1), and (C) chloroform–acetic acid (50:0.1). The solvent was allowed to rise to about 12 cm above the origin (10–12 min), then the chromatograms were removed, and air-dried for 5–10 min.

Barbiturates were detected by spraying chromatogram C with diphenylcarbazone until a uniform pink color persisted. This was allowed to dry (7–10 min), then sprayed with mercurous nitrate. The barbiturate appeared as a white spot on a blue background that developed in about 5 min.

Chromatograms A and B were viewed under ultraviolet (UV) light for fluorescence due to quinine and meperidine. Chromatogram B was then exposed to ammonia vapors for 30 sec and sprayed with Fluoram. Under UV light, *d*-amphetamine and quinine appear as yellow spots. Both chromatograms A and B were then sprayed with iodoplatinate to detect opiates, opioids and other basic drugs.

TLC spray reagents

Iodoplatinate stock solution consisted of 5 ml of 5% (w/v) platinum chloride, 45 ml 10% (w/v) KI and 45 ml water. Prior to use the iodoplatinate stock solution was mixed with an equal volume of 2 *M* HCl. Diphenylcarbazone was prepared as a 1% w/v solution in 95% ethanol. The mercurous nitrate solution consisted of 20 g $HgNO_3 \cdot H_2O$ in 500 ml of 0.15 *N* HNO_3. The Fluoram spray, used for detection of primary amines, consisted of 15 mg of fluorescamine in 100 ml of acetone.

Gas–liquid chromatography

Drugs were extracted from 5 ml of urine using the method of Goldbaum *et al.*[10]. GLC analyses of these extracts were performed on a Hewlett-Packard Model 5700 dual-column gas chromatograph fitted with hydrogen flame detectors. The glass columns were 3 ft. × ¼ in. O.D., packed with 3% OV-1 or 3% OV-17 absorbed on 80–100 mesh Chromsorb W HP. Column conditions are as stated in Table IV. The injection port temperature was 250° and the detector temperature 300°. The respective flow-rates for helium, hydrogen and air were 60, 60 and 240 ml/min, respectively. An injection volume of 2 μl was used and absolute retention times were measured from the front edge of the solvent peak.

Radioimmunoassay

The morphine RIA was ^{3}H-labelled, whereas the barbiturate and amphetamine RIAs used ^{125}I for the radioactive label. The RIA kits were used according to the method outlined by the manufacturer, and 0.1 ml of urine was allowed to incubate with the antibody solution for 1 h[21]. After precipitation of the antibody–drug complex, 0.5 ml of the supernatant was added to 10 ml of modified Bray's solution and counted for 1 min in the tritium channel of a Packard Tri-Carb Model 3330 liquid scintillation spectrometer. Both ^{3}H and ^{125}I can be counted in this manner.

A control urine, as well as three standard urines containing known amounts of drugs, were run for each different drug. The standard urines for morphine, barbiturate and amphetamine contained 60, 100 and 1000 ng/ml of the respective drug. A patient's urine was considered positive on RIA for the drug in question if the counts obtained were greater than the mean count obtained for its respective standard urine.

RESULTS

The extraction efficiency of the Amberlite XAD-2 resin, using the plastic bag column and methanol elution, was determined using normal urine spiked with radioactive drugs. Efficiencies for morphine, barbiturate, amphetamine and methadone are shown in Table I. The flow-rates expressed in Table I represent maximum flow-rates for the bag-type column. Attempts to study the effects of increased flow-rates by increasing the diameter of the column outlet resulted in a loss of Amberlite XAD-2 resin through the outlet. The effect of decreased flow-rate on drug extraction efficiency was studied by decreasing the diameter of the column outlet. Use of an 18-gauge needle to make the outlet hole resulted in flow-rates of 4.3 ml/min for urine and 2.6 ml/min for methanol. While the per cent recovery of all drugs increased slightly, the time required for the extraction process of a single sample doubled from 10 to 20 min. Because of this substantial increase in extraction time, the higher flow-rates were employed for all subsequent studies.

As shown in Table I, increase in urine pH from 6.2 to 8.1 increased the per cent recovery of morphine and amphetamine only slightly, whereas the recovery of phenobarbital in the methanol eluate decreased to 45%. We have found that patient urine

TABLE I

EXTRACTION EFFICIENCY OF AMBERLITE XAD-2

50-ml urine samples were spiked with each of the ^{14}C-labelled drugs at a concentration of 1 μg/ml. 1-μCi total radioactivity was used in each extraction. Each value represents the mean of at least three separate determinations. Flow-rates for urine and methanol through the column were 8 and 4.7 ml/min, respectively.

Drug	*Extraction at pH 6.2 (%)*	*Extraction at pH 8.1 (%)*
Morphine	83.3	88.8
Methadone	88.5	—
Phenobarbital	81.4	44.9
Amphetamine	80.7	81.3

pH just prior to analysis (2 to 12 h after the urine sample is taken) normally ranges from pH 5.2–7.5 with a mean pH of 6.2. Urine pH, therefore, was not changed prior to extraction, since the lower pH favored efficient extraction of barbiturates.

Thin-layer chromatography

Table II shows the R_F and the color of the spot for several drugs separated by the three different solvent systems. These represent compounds most frequently encountered in the urine of patients being treated for drug abuse. While only one spot at R_F 0.5 is listed for codeine, a second spot at R_F 0.15 (morphine) is usually seen in the urine of a patient who has taken codeine. Similarly, we have occasionally observed two spots in urine from a patient who has taken heroin, a major morphine spot at R_F 0.15 and a spot at R_F 0.8 corresponding to 6-acetylmorphine. Because of this appearance of multiple spots for certain drugs, the standard urines used for comparison on TLC were obtained from patients who had received the given drug rather than using urine from normal subjects spiked with the drug.

TABLE II

R_F VALUES AND SPOT COLORS OF DRUGS SEPARATED BY TLC

DPC = Diphenylcarbazone spray followed by mercurous nitrate spray as described in the text. B = Blue; Bl = black; Br = brown; Gr = gray; Or = orange; Pp = purple; R = red; W = white; Y = yellow; dk = dark; Fl = fluorescent under short-wave UV light.

Drug	*R_F in solvent system*			*Spot color*		
	A	*B*	*C*	*Iodoplatinate spray*	*Fluoram spray*	*DPC*
Codeine	0.5, 0.15	0.1		B		
Hydromorphone	0.5	0.1		dk Pp		
Morphine	0.15			B		
Heroin	0.15, 0.8	0.4		B-Bl		
Methadone	1.0	0.5		Br		
Meperidine	0.75			Pp		
Propoxyphene·HCl	0.2–1.0	0.3		B		
d-Amphetamine	0.85	0.4		Gr	Y/Fl	
Methamphetamine	0.85	0.4		Gr		
Cocaine	1.0	0.65		Pp-Br		
Benzoyl ecgonine	0.4			Pp		
Caffeine	0.8	0.8		Gr		
Procaine	0.9	0.2		Gr		
Pentobarbital			0.7			Pp-W
Amobarbital			0.7			Pp-W
Secobarbital			0.7			Pp-W
Phenobarbital			0.7			Pp-W
Meprobamate	0.15			W		
Methyprylon		0.8		Y-Or		
Ethinamate	....		0.7			Bl
Desipramine	0.2			Pp		
Hydroxyzine·HCl	0.1	0.1		dk Br		
Chloral hydrate	0.2	0.5		B		
Thioridazine	0.1–0.6	0.5		Br		
Chlorpromazine	0.8	0.7		B		
Quinine	0.3	0.3		Bl		
Mandelamine	0.5			R		

For certain basic drugs listed in Table II, characteristic spots on TLC were not observed with both solvent systems A and B. In these cases, either drug spots were obscured by urinary pigments or the drug did not migrate from the origin of the chromatogram. In addition, a drug such as propoxyphene tends to streak in solvent system A, whereas it separates as a defined spot in solvent system B.

The sensitivity of the TLC procedure was estimated using normal urine spiked with morphine, methadone, barbiturates, and amphetamines. Table III shows the results of analysis of 130 urine samples spiked with mixtures of the above drugs at concentrations of 1.0, 0.5, or 0.2 μg/ml. Analysis of these urine samples was performed in a single blind manner in which the technicians performing the test did not know the identity or amounts of drugs present. The increase in false-negative results with decreasing concentrations of a given drug was used to estimate the lowest concentration of that drug which could be routinely detected by TLC. No false negatives were observed for morphine, and only one questionable positive was noted for morphine at the lowest concentration (0.2 μg/ml). For methadone, the incidence of false negatives was high at 0.2 μg/ml, and low at 0.5 μg/ml. The estimated sensitivity for detecting barbiturates is between 0.5 and 1.0 μg/ml and about 0.5 μg/ml for *d*-amphetamine. For methamphetamine, however, the sensitivity of the TLC assay is not as good, since we observed about 50% false negatives at the highest concentration studied (1 μg/ml).

Radioimmunoassay

Radioimmunoassay was used for confirmation of TLC results for morphine and barbiturates. The amphetamine RIA, at present, is still being evaluated as a con-

TABLE III

TLC ANALYSIS OF 130 NORMAL URINES SPIKED WITH DRUGS

Some urine samples were spiked with more than one drug. All chromatograms were interpreted by the same person.

Drug	*Total number of urines*	*Concentration (μg/ml)*	*Negative*	*Positive*	*Questionable*	*Approximate sensitivity (μg/ml)*
Morphine	23	1.0	—	10	—	0.2
		0.5	—	8	—	
		0.2	—	4	1	
Methadone	38	1.0	2	17	1	0.5
		0.5	1	9	1	
		0.2	2	2	—	
Barbiturates	51	1.0	2	20	2	0.5–1.0
		0.5	8	9	2	
		0.2	7	1	—	
d-Amphetamine	30	1.0	2	15	—	0.5
		0.5	—	7	1	
		0.2	1	2	1	
Methamphetamine	20	1.0	6	7	—	>1.0
		0.5	3	2	—	
		0.2	2	—	—	

firmatory procedure. It should be noted that the amphetamine RIA is specific for *d*-amphetamine and does not react with methamphetamine.

Gas–liquid chromatography

For GLC analysis, a multi-extraction procedure was used in which the drugs are separated into three fractions: acid, basic and neutral drugs[10]. The basic fraction contains opiates, methadone, amphetamines, and cocaine; the acid fraction contains barbiturates. The neutral fraction contains drugs such as glutethimide. Since morphine is amphoteric, it is not extracted by this procedure and remains in the aqueous layer. For this reason, and because morphine cannot be chromatographed well by GLC, confirmations of morphine were performed using RIA.

The extraction efficiencies for the different drugs vary using the above procedure. However, the high sensitivity of GLC compensates to give a procedure that is more sensitive than TLC for all the drugs tested. Table IV lists retention times for each drug; included are the metabolites of some drugs which are found in actual patient urine samples.

TABLE IV

RETENTION TIMES AND COLUMN TEMPERATURES FOR GLC ANALYSIS OF URINE FOR ABUSIVE DRUGS

Retention times are relative to the leading edge of the solvent front and were measured directly by an automatic integrator. EDDP = 2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine; EMDP = 2-ethyl-5-methyl-3,3-diphenylpyrroline.

Drug	*Retention time (min)*		*Column temperature (°C)*
	3% OV-1	*3% OV-17*	
Methadone		5.34	240
Methadone	0.88		250
EDDP		4.00	240
EDDP	0.72		250
EMDP		3.04	240
Codeine	1.54		250
Darvon		5.90	240
d-Amphetamine		1.95	140
Methamphetamine		2.43	140
Amobarbital	0.93		200
Pentobarbital	0.97		200
Secobarbital	1.20		200
Phenobarbital	2.05		200
Quinine	4.60		250
Cocaine		8.28	240
Cocaine	1.26		240
Cocaine	0.97		250
Benzoyl ecgonine	3.15		240

DISCUSSION

The sensitivity of the TLC method in detecting abusive drugs in urine is important because, as used here as the initial analysis, it determines the ultimate sensi-

tivity of the urine-testing program. The sensitivity of the TLC method is related to the volume of urine used, the efficiency of the extraction of the drug from urine, and the sensitivity of the chromatography system.

Drugs can be extracted from urine using liquid–liquid extraction[11], ion-exchange resin[12] or absorption resins[9,13,14]. In the present study, we used an absorption resin column method originally developed in this laboratory[9,13]. This method uses Amberlite XAD-2, a non-ionic resin, to absorb water-soluble organic molecules (drugs) from urine. Compared to liquid extraction procedures or ion exchange, the Amberlite XAD-2 procedure is faster, more efficient, and able to extract a wider variety of drugs. The efficiency with which XAD-2 will extract drugs from urine depends on several factors. Kullberg *et al.* and Miller *et al.* have shown that the rate of urine flow through an Amberlite XAD-2 column influences extraction efficiency, and both authors have used elaborate devices to regulate this flow[15,16]. In addition, these authors have shown that extraction efficiency is also dependent on the rate of flow of the eluting solvent. The column used in the present study was designed to be inexpensive and disposable. Flow-rate is controlled simply by the size of the outlet hole in the plastic bag column (Fig. 1). Respective flow-rates for urine and eluting solvent of 8 and 4 ml/min resulted in extraction efficiencies of greater than 80% for the four test drugs. While the extraction efficiencies could be increased slightly by decreasing the flow-rate by 50%, the time necessary to extract the drugs from urine was doubled, thus the higher-flow-rate column was used in order to keep extraction time at a minimum.

The pH of the urine has also been shown to affect extraction efficiency[15,16]. In the present study, we found that we could increase the extraction efficiency for morphine by only about 5% if the urine were buffered to pH 8.1 prior to passage through the XAD-2 column (Table I). At a urine pH of 8.1, however, less than 50% of phenobarbital was extracted. It was decided, therefore, to use the urine as it was received, since few urines above pH 7.5 were encountered and the mean urine pH was about 6.2.

The extraction solvent used to elute drugs from XAD-2 has been the subject of some concern. Several different extraction solvents have been reported including chloroform–isopropanol[14], ethylene dichloride–ethylacetate[16], methanol–chloroform[17], and methanol[9,13]. Elution with non-polar solvents results in an extract that contains fewer interfering substances than elution with a polar solvent such as methanol. However, Kullberg *et al.* have shown that extraction efficiencies with non-polar solvents are lower unless very slow flow-rates of eluting solvents are used (2 ml/min or less)[15]. Furthermore, with non-polar solvents an additional step is necessary to separate the organic phase from the aqueous phase.

The TLC method employs three different solvent systems. Others have used a single solvent system and sprayed the chromatogram sequentially with several reagents which develop a color with different drugs[14]. Because of the difficulty and possible errors in reading such a chromatogram, we used three different solvent systems with a less complex spray system. Solvent system C is used to separate barbiturate and solvent systems A and B are used to separate basic drugs. The R_F values of the different drugs are shown in Table II. To detect barbiturates on the chromatogram developed in solvent system B, we have used the sequential spray consisting of diphenylcarbazone oversprayed with mercurous nitrate as described by Wang and Mueller[13]. Basic drugs such as opiates and opioids are detected on solvent system B

sprayed with iodoplatinate. Amphetamines are detected on solvent system B by spraying with Fluoram and observing under UV light. This chromatogram is then oversprayed with iodoplatinate as a cross-check for the basic drug observed with solvent system A. With this spray procedure the technician has time to spray a number of chromatograms and interpret in groups rather than individually.

While the drugs listed in Table II do not represent all the drugs that can be identified using the above TLC method, they do represent compounds most commonly encountered in the urine of drug abuse patients.

The TLC method described is used as the initial method of determining the presence or absence of illicit drugs in the urine of patients receiving treatment for drug abuse. The more sensitive methods, RIA and GLC, are used only for confirmation of TLC results. In terms of sensitivity, we have found that, in general, TLC can detect the presence of heroin (as morphine), barbiturates, amphetamines and methadone for at least 36 h after a patient has taken one of these drugs. For example, in a recent study on barbiturates in our laboratory, TLC could detect barbiturate in 90% of the urine samples tested for up to 30 h after a subject ingested 100 mg of barbiturate. At 48 h, barbiturate was detected in about 70% of the urine samples tested[18]. This represents about 10% false-negative results for the first 30 h after ingestion and 30% false-negative results at 48 h. In drug treatment programs where urine samples are obtained twice weekly on a random schedule, any patient abusing drugs will not go undetected for very long.

Unfortunately, TLC produces a significant proportion of false-positive results which mislead the physician regarding the patient's progress and status in the drug treatment program. For example, of 130 urines analyzed in Table III, a certain number of these urines (data not shown in Table III) were found positive for drugs they did not contain. There were seven false positives for methadone, one for morphine, three for barbiturates and two for amphetamines using TLC. These false-positive results can occur due to interference by other drugs, interference from non-drug substances or nonspecific factors that make the chromatogram difficult to interpret. It is because of such false-positive results that other methods are needed to confirm results obtained by TLC.

GLC has been used as a confirmatory method, since it is more sensitive than TLC, has greater separation ability, and is less prone to false-positive results. Unfortunately, the increased time for analysis and greater cost of GLC have made it less attractive than TLC as a primary method for urine surveillance.

In routine use, urines found positive or questionably positive for barbiturates, amphetamines, methadone or cocaine (using TLC) are analyzed a second time by GLC. In addition, certain urine samples which show obvious interference for a given drug in the TLC system are subsequently analyzed by GLC. For example, the positive and questionably positive TLC results shown in Table III (except for morphine results) were confirmed as positive by subsequent analysis on GLC. Most important, however, the false-positive results previously mentioned (except for morphine) that were obtained with TLC were found to be negative with GLC. In this instance, therefore, in the 130 urine samples analyzed, confirmation of positive TLC results by GLC reduces false-positive results to nearly zero. In addition to the elimination of false-positive results, confirmation by GLC of questionably positive TLC results can slightly increase the sensitivity of the urine testing. Of the 130 urines tested, one false posi-

tive for methadone was observed with GLC; however, this sample was not positive with TLC.

More recently, immunoassay techniques have become available for certain abusive drugs. While the antibodies exhibit a high degree of specificity, they do react with some analogs of the parent drug. For example, antibodies produced to react with morphine react equally well with codeine. Because of such cross-reaction, the immunoassay procedures for abusive drugs cannot be relied upon as absolute evidence of a drug's presence, but only as an indication. Furthermore, due to the high sensitivity of most immunoassay methods for abusive drugs, confirmation by another method is difficult. For these reasons, we have used immunoassay for confirmation of TLC results rather than as a primary method for urine surveillance.

At present there are four types of immunoassay procedures for abusive drugs: (1) RIA, (2) enzyme multiplied immunoassay technique, (3) free radical assay technique, and (4) hemagglutination inhibition. Of these four types, we have been using RIA because it is the most sensitive and has the capability of being used quantitatively[19]. The morphine RIA has proven particularly useful since morphine cannot be easily confirmed by GLC. While codeine and other morphinans are detected with the morphine RIA, this presents no great problem since TLC can distinguish morphine from codeine. In normal practice, RIA is used to confirm all positive and questionably positive results for morphine observed with TLC. When large amounts of quinine are present (a condition which often obscures the morphine spot) RIA for morphine is routinely run on these samples. In addition, when diluted urine samples are received (specific gravity 1.005 or less) RIA for morphine is routinely performed. However, in the latter two cases, positives for opiates are reported as unconfirmed analyses.

In addition to the morphine RIA, we have studied the use of a barbiturate RIA for the confirmation of positive or questionable results for barbiturates obtained with TLC[18]. The results of this study have shown the barbiturate RIA to be an excellent method of confirmation; it is as sensitive as GLC, faster and less expensive. In contrast to GLC, the barbiturate RIA does not distinguish between the different barbiturates. Preliminary data have shown the barbiturate RIA to cross-react with glutethimide; however, this poses no problem since glutethimide can be distinguished from barbiturates on TLC[13].

More recently, we have been evaluating an amphetamine RIA for confirmation of positive or questionably positive amphetamine results obtained with TLC. The sensitivity of the amphetamine RIA is only 1000 ng/ml urine compared with 60 and 100 ng/ml for morphine and barbiturate RIAs, respectively. Present evidence suggests that the amphetamine RIA will also be a useful confirmation method.

The use of RIA for confirmation purposes has the advantages over GLC in that it is faster and, when confirming the presence of a single drug, less expensive. Furthermore, RIA can be performed by a technician with a minimum of training, which greatly facilitates the incorporation of RIA into existing urine surveillance methods.

In conclusion, TLC used as a primary method for urine surveillance is sufficiently sensitive to produce clinically meaningful results for use in the treatment of drug abuse. TLC has the advantage of simultaneously analyzing several drugs at a moderate cost. However, the possibility of false-positive results is greater with TLC than with other methods, and the need for high reliability dictates the use of con-

firmatory procedures. Confirmation of TLC results by GLC and RIA reduces the possibility of false-positive results to nearly zero and results in a high overall reliability for the combined urine surveillance method. These two confirmation methods are equally sensitive. GLC is, however, more versatile since it can detect a wider range of drugs, while RIA is faster and less expensive. Presently, RIAs are available for only morphine, barbiturates and amphetamines. As RIAs for other drugs of abuse become available it is expected that they can be easily integrated into present urine surveillance methods for confirmation purposes.

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