J Forensic Sci, Apr. 1976, Vol. 21, No. 2

E. R. Noe,[1] *M.S.; G. D. Lathrop,*[1] *M.D., Ph.D.;*
C. A. Ainsworth III,[1] *M.S.; and J. H. Merritt,*[1] *B.S.*

Radioimmunoassay Technology in Mass Drug Screening: An Evaluation of an Absorbent Paper Disk Transport System

The military screening program for the drugs of abuse requires the shipment of approximately 3 million urine specimens annually to regionalized testing laboratories in the United States, Europe, and Southeast Asia. Shipments are made by parcel post, air freight, bus, and other forms of ground transportation, incurring considerable expense. The space required for storing these specimens during the processing and reporting period creates a logistical problem due to the bulk of the material being handled. Based on this, a method for transporting urine specimens for the military that would obviate the time and expense incurred in shipping large volumes of urine was highly desirable.

Until recently, the most common methods used for drug screening were based on thin-layer and gas-liquid chromatography. These procedures required the use of relatively large volumes of urine. With the development of a highly sensitive, relatively specific, semiautomatic radioimmunoassay (RIA) mass screening procedure for amphetamines, barbiturates, and opiates, it is now possible to detect nanogram quantities of these drugs in 30 μl volume [*1,2*].

This paper is an evaluation of a transport system using absorbent paper as the urine-drug vehicle. The shipment of biological fluids by this method is not new [*3,4*], and in practice it has been very effective. Preliminary studies by a proprietary research laboratory under the auspices of the military demonstrated the possibility of using urine-impregnated disks in the RIA testing methodology.[2]

Materials and Methods

Specimen Collection and Preparation

Pre-numbered 2 by 7-cm strips of Schleicher and Schuell (S&S) filter paper No. 470C were saturated by thorough immersion in the urine specimen for 2 to 4 s. Strips were handled with forceps and transferred to a stainless steel wire rack for drying at room temperature. Two to four hours were required for complete drying; the variability was largely due to the ambient relative humidity. The strips may also be dried in a 37°C

Received for publication 23 June 1975; accepted for publication 25 Aug. 1975.

[1] Chief, Drug Abuse Detection Branch; Chief, Epidemiology Branch; Chief, Toxicology Section; and research chemist, respectively; USAF School of Aerospace Medicine, Brooks AFB, Tex.

[2] Personal communication with E. Grunberg and R. Cleeland, Roche Diagnostics, Nutley, N.J., 1974.

(98.6°F) incubator for approximately 45 min. When the strips are stacked for shipment to a toxicology laboratory for analysis, it is essential that they be completely dry, otherwise cross-contamination of the strips may occur. Alternatively, multiple test strips can be shipped in individual paraffin envelopes. When the strips are processed, disks with individual diameters of 6.5 mm are punched out using an ordinary commercial hand punch. A total of 25 disks may be punched from each strip. These disks are transferred directly into an appropriately labeled 12 by 75-mm polystyrene test tube. All materials used in preparing the disks are shown in Fig. 1.

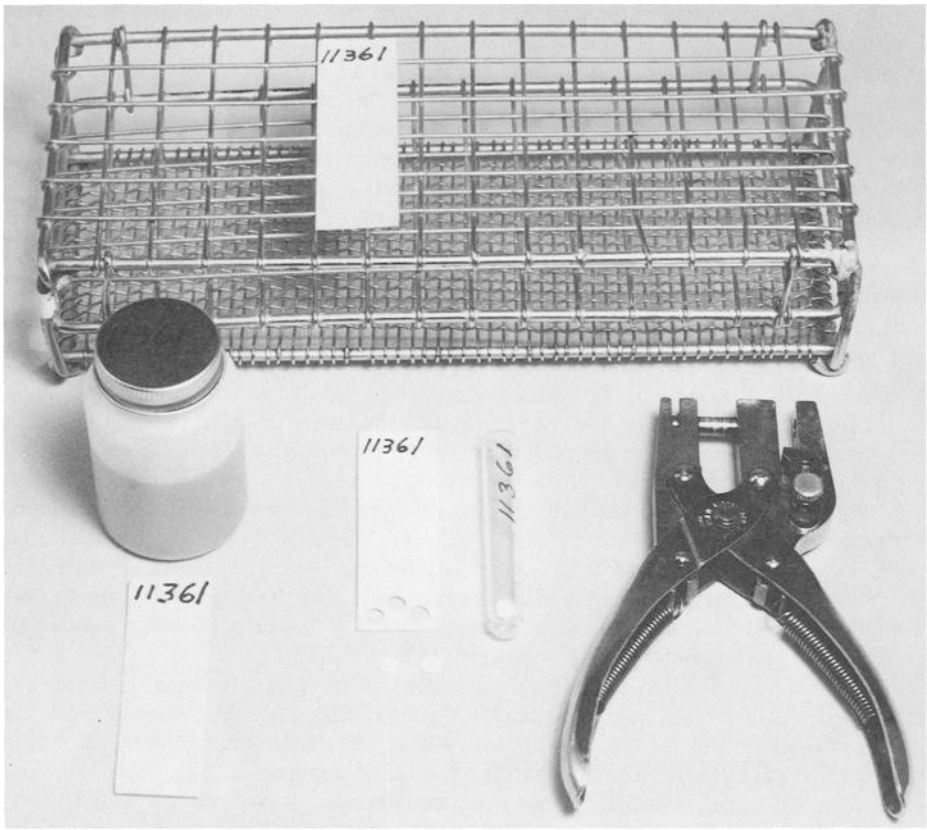

FIG. 1—*Materials used in preparing disks.*

Reagents

The RIA kits for bivalent morphine-barbiturate and for monovalent morphine, amphetamine, and barbiturate were procured from a commercial source.[3] The kits were prepared for use according to the instructions provided by the manufacturer.

A saturated ammonium sulfate and distilled water were the only other reagents required. In preparing the ammonium sulfate, a few crystals were allowed to settle to the bottom of the reagent bottle. Pooled RIA negative urine was used to prepare spiked morphine, barbiturate, and amphetamine standards.

[3] Hoffman-LaRoche, Inc., Nutley, N.J.

Procedure

Spiked urine standards containing exact amounts of amphetamine, barbiturates, and morphine were prepared and assayed in a single-blind fashion. Both standard and unknown urine specimens were processed through the RIA test system [*1,2*] for the specific drug under test. Since a paper disk containing urine residue was used in place of liquid urine, a Micromedic® autopipeting station was set to add 0.5 ml of the RIA reagent mix. After reagent addition, the tube racks must be agitated by hand shaking for approximately 15 s to insure elution of the drugs from the paper disk. Erratic results will occur if the shaking step is omitted. After a 1-h incubation at room temperature, the racks were transferred to the second automatic pipeting station. Saturated ammonium sulfate was added and the racks were shaken again and then allowed to set for 10 min. The volume of ammonium sulfate was increased from the 0.5 ml called for when using urine directly to 0.7 ml to preclude "carryover" problems [*2*]. The supernatant fluid (0.5 ml) was removed from the reaction mixture and transferred to a clean polystyrene test tube, followed by a 1-ml wash, again using an automatic pipetor. The gamma emission of the ^{125}I drug released during the equilibrium reaction was measured in a Searle Model 1285 gamma counter. All specimens were counted for 0.4 min.

Results and Discussion

Fifty 6.5-mm disks punched from S&S 470C filter paper strips were weighed before and after saturation with urine having an average specific gravity of 1.015. The disks were immersed in urine with forceps for 4 s, shaken gently, and weighed immediately on an analytical balance. The average volume absorbed was calculated to be 31.8 μl with one standard deviation being 3.1 μl. This variation is satisfactory for drug screening by the sensitive RIA procedure.

In the barbiturate RIA procedure, the relative cross-reactivity of pentobarbital, amobarbital, and phenobarbital is 0.39, 0.15, and 0.08, respectively, against a secobarbital reactivity of 1.0. This is in good agreement with the cross-reactivity found by Mulé et al [*5*]. There is no cross-reactivity between amphetamine and the other two drug classes, morphine and barbiturates. The same is true for each of the drug classes in relation to each other. Typical standard curves are shown in Figs. 2–4.

A single-blind evaluation of the paper disk method for RIA barbiturate analysis was conducted and the results are presented in Table 1. The data are consistent with the cross-reactivity known for the barbiturates. Within the framework of the military drug testing program, detection of phenobarbital is of little importance. Phenobarbital abuse within the services is essentially nonexistent, and laboratory positives are usually associated with prescribed medications containing phenobarbital. The data for morphine and amphetamine absorbent disks are shown in Table 2. The data show that the morphine RIA reagent is the most sensitive of the three drug class reagents and that morphine can be detected at very low levels. The problem associated with a cutoff level of under 200 ng/ml of morphine is that of confirming its presence by another technique. It is noteworthy that no false positive results occurred in the study.

Table 3 shows fine correlation between blind parallel disk and urine assays. Coded parallel disk and urine specimens were submitted to the Brooks Air Force Base drug detection laboratory from a local military drug abuse rehabilitation center on a blind technique basis. Comparisons of disk and urine results were made after all analyses were completed, and the coding key was supplied by the rehabilitation center. In addition to routinely submitted urine specimens, the center also randomly inserted spiked specimens of each drug class supplied by our quality control (QC) section. These specimens were

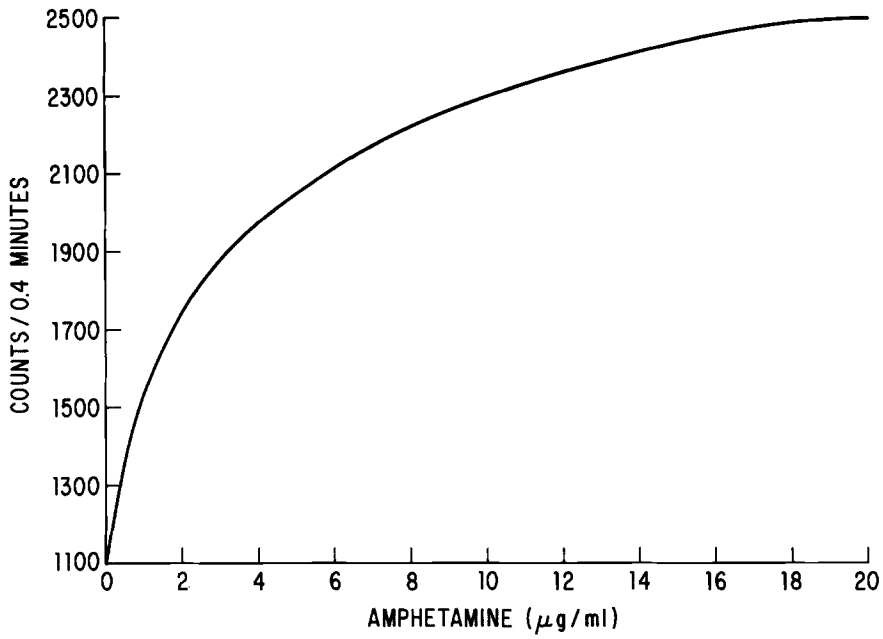

FIG. 2—*Concentration curve for amphetamine. Standards were added to pooled negative urine and diluted to the concentrations indicated. Filter paper strips were dipped in their respective standard concentration, dried, and one 6.5-mm disk punched from each concentration strip used in the procedure.*

spiked with morphine, secobarbital, and amphetamine at concentrations of 500, 1000, and 5000 ng/ml, respectively, and accounted for the majority of the urine-positive group.

The double-blind study was programmed to evaluate over 10 000 specimens; however, only 736 paired urine and filter paper strip specimens were studied before the suspension of the military drug abuse testing program in July 1974. The program resumed in March 1975, and a further evaluation is planned in the near future.

One major problem with using filter paper strips as the vehicle for shipping urine was discovered and corrected early in the double-blind study: the diffusion of drugs between adjacent filter paper strips. Morphine, barbiturates, or amphetamines diffused to other strips when the drying process was not completed before shipment.

To verify this problem, we initiated a diffusion study. One hundred and twenty strips were processed and dried with morphine, barbiturate, and amphetamine. Twenty of each drug class were processed until they were just damp to the touch, then they were alternated with negative urine strips in stacks. The dry drug strips were likewise alternated with dry negative-urine strips in stacks. All stacks were set aside and at the end of seven days were processed by RIA. Table 4 shows the tabulated results of the study. From the data obtained it is obvious that contamination from a negative strip stored adjacent to a strip containing a drug under test does occur unless all strips are completely dry.

The dry standard strips stored at room temperature used in the diffusion studies were subsequently tested an additional four times at weekly intervals. Amphetamine, barbiturate, and morphine absorbed on these strips showed no demonstrable loss of activity in their respective RIA test systems. The slight decrease in specific activity noted was due to the radioactive decay of ^{125}I.

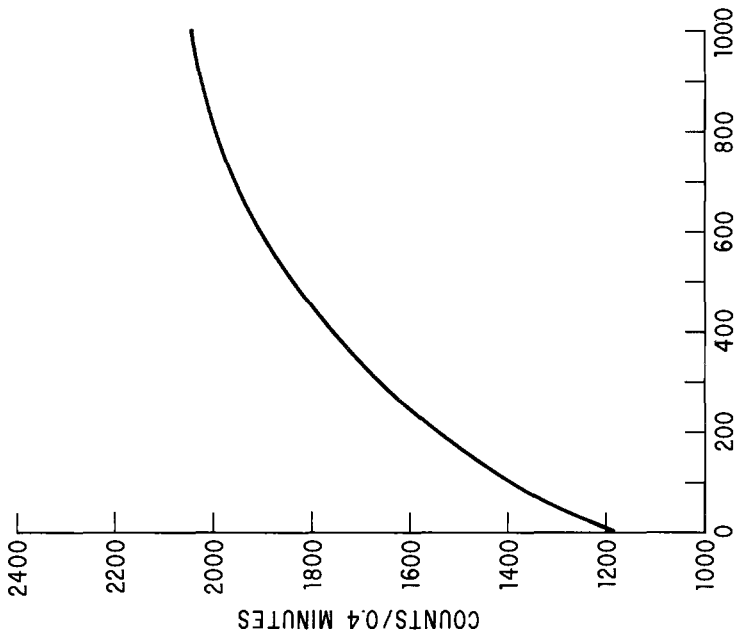

FIG. 4—*Concentration curve for morphine. Standards were added to pooled negative urine and diluted to the concentrations indicated. Filter paper strips were dipped in their respective standard concentration, dried, and one 6.5-mm disk punched from each concentration strip used in the procedure.*

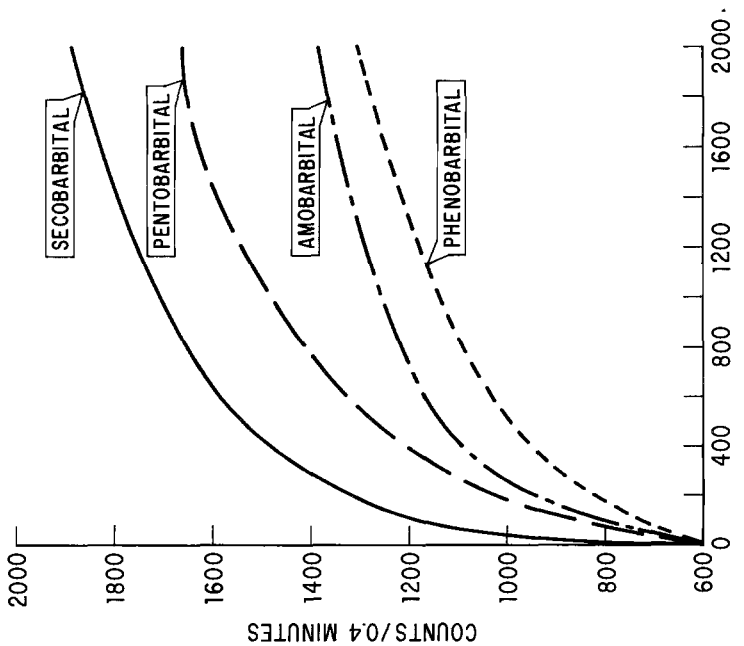

FIG. 3—*Concentration curve for barbiturates. Standards were added to pooled negative urine and diluted to the concentrations indicated. Filter paper strips were dipped in their respective standard concentration, dried, and one 6.5-mm disk punched from each concentration strip used in the procedure.*

TABLE 1—*Single-blind evaluation on barbiturate-absorbent disks.*

Concentration, ng/ml[a]	Specimens, no.[b]	Rated Positive, no.	Correct, %
	Secobarbital		
100	32	3	9.3
250	32	15	46.8
500	32	25	78.1
750	29	28	96.5
1000	32	32	100
1500	32	32	100
2000	32	32	100
	Pentobarbital		
250	32	4	12.5
500	32	9	28.1
750	32	29	75
1000	32	29	90.6
1500	32	30	93.7
2000	16	15	93.7
	Amobarbital		
250	32	5	15.6
500	32	3	9.4
750	32	8	25.0
1000	32	14	43.7
1500	32	29	90.6
2000	32	29	90.6
	Phenobarbital		
250	32	0	0
500	32	0	0
750	32	0	0
1000	32	5	15.6
1500	32	10	31.2
2000	32	13	40

[a] RIA cutoff level for barbiturates was set at 200 ng secobarbital/ml.
[b] This study included 746 interspersed specimens negative for barbiturates, and no false positives were detected.

Summary

These studies have demonstrated the feasibility of using urine-saturated paper disks in place of urine in the RIA system for drug abuse detection. Results with the disks are consistent with those using urine. A satisfactory procedure has been devised which provides reproducibility of results with no loss of sensitivity or specificity. Further, the procedure is essentially the same as the current procedure requiring urine except that a paper disk punched from a filter paper strip impregnated with urine is used. Complete flexibility is retained to switch from urine to disk. No new or additional equipment is required.

It is envisioned that the urine would remain at the collection site and dried filter paper strips containing urine under test be shipped to toxicology laboratories. Should the disk assay be positive, the urine specimen identified with that disk could then be shipped to the laboratory for confirmation by gas-liquid chromatography or other acceptable methods. The time and expense incurred in shipping large volumes of urine would thus be eliminated.

TABLE 2—*Single-blind evaluation on morphine- and amphetamine-absorbent disks.*

Concentration, ng/ml[a]	Specimens, no.[b]	Rated Positive, no.	Correct, %
	Morphine		
100	80	20	25.0
200	72	70	96.2
250	72	69	97.3
500	80	79	98.7
750	38	38	100
1 000	38	38	100
2 000	29	29	100
	Amphetamine		
1 000	48	23	47.9
2 000	48	42	87.5
3 000	47	43	91.5
4 000	90	86	95.5
5 000	48	46	95.8
7 500	28	28	100
10 000	17	15	88.2
20 000	11	10	90.9

[a] RIA cutoff levels for morphine and amphetamine are 200 and 2000 ng/ml, respectively.

[b] This study included 781 interspersed specimens negative for both morphine and amphetamine, and no false positive results were noted.

TABLE 3—*Parallel blind study: comparison of absorbent disk and urine test results.*

Drug[a]	Disk Positives, no.	Urine Positives, no.[b]	Correct (Disk/Urine), %
Morphine	43	47	91
Barbiturate	45	45	100
Amphetamine	35	40	87.5
Negative specimens (604)	0	0	100

[a] RIA cutoff levels were 200, 200, and 2000 ng/ml for secobarbital, morphine, and amphetamine, respectively.

[b] Coded spiked specimens sent by a rehabilitation center, interspersed among routine specimen submissions.

TABLE 4—*Diffusion study.*

Drug[a]	Strips, no.	Dry/Dry Counts, avg.	Dry/Damp Counts, avg.
Amphetamine	20	2270	2180
Negative	20	1380	2050
Morphine	20	2550	2470
Negative	20	1540	1750
Barbiturate	20	1575	1300
Negative	20	550	750

[a] Drug levels were 500, 1000, and 4000 ng/ml for morphine, secobarbital, and amphetamine, respectively.

Acknowledgments

The authors want to thank Louie G. Brewer, Jr. and William A. Romanchick for professional advice rendered. They also wish to express their appreciation for the outstanding technical assistance provided by Kerry A. Luke, Robert D. McKelvey, Robert R. Ibarra, Gabriel Hernandez, Ronald T. Wilber, John L. Wenck, and Paul M. Byrnes during this evaluation. In addition, the assistance of personnel assigned to the Army Drug Abuse Collection Center at Ft. Sam Houston, San Antonio, Tex., was greatly appreciated.

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G. D. Lathrop, M.D., Ph.D.
Epidemiology Division
USAF School of Aerospace Medicine
Brooks AFB, Tex. 78235