J Forensic Sci, Jul. 1978, Vol. 23, No. 3

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Detection of Barbiturates by Latex Agglutination Inhibition

Spector and Flynn [*1*] initially demonstrated that antibodies to barbiturates could be elicited by immunization of rabbits with a secobarbital derivative coupled to bovine serum albumin. The resultant antiserum and a ^{14}C- or ^{125}I-labeled barbiturate derivative were used to develop a sensitive and specific radioimmunoassay [*1–3*]. Although the radioimmunoassay obviated the need for any sample pretreatment, it did require a nuclear counter and the use of radioactive materials. An enzyme-coupled immunoassay [*4*] and a hemagglutination inhibition method [*5*] have been reported as alternative approaches for the detection of barbiturates.

A test using a barbiturate derivative coupled to latex particles as the carrier for an agglutination-inhibition reaction was evaluated in this study. In principle, when barbiturate-coupled latex is added to a specific antiserum in the absence of any other barbiturate, the resulting antigen-antibody reaction causes visible aggregation of the latex and is designated a negative result. In the presence of relatively large amounts of barbiturate, the antigen-antibody interaction is then primarily between the specimen barbiturate and the antiserum. Consequently, aggregation of the barbiturate-coupled-latex particles does not occur and the solution remains clear and translucent. Variations in barbiturate concentration consequently produce variations in the degree of agglutination.

This report describes the preparation, development, and application of a latex agglutination-inhibition tube test for the detection of barbiturates in urine and serum.

Materials and Methods

Reagents

Tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) buffer, pH 7.3, was prepared by dissolving 15.7 g of 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloride (Schwarz-Mann, Orangeburg, N.Y.) in distilled water containing 0.85% sodium chloride, adjusting the pH to 7.3 ± 0.1 with 10% sodium hydroxide, and diluting to 1 litre.

Morpholinoethanesulfonic acid (MES) buffer, pH 6.5, was prepared by dissolving 19.5 g of 2(*N*-morpholino)ethane sulfonic acid (Sigma Chemical, St. Louis, Mo.) in distilled water containing 0.85% sodium chloride, adjusting the pH to 6.5 ± 0.1 with 10% sodium hydroxide, and bringing the total volume to 1 litre.

Presented in part at the 29th Annual Meeting of the American Academy of Forensic Sciences, San Diego, Calif., February 1977. Received for publication 28 Oct. 1977; accepted for publication 12 Dec. 1977.

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Rabbit antibarbiturate serum was produced according to the procedure of Spector and Flynn [*1*]. The antiserum was diluted with Tris-HCl buffer, pH 7.3, containing 0.1*M* ethylenediaminetetraacetic acid (EDTA) so that 300 to 500 μg/litre of secobarbital inhibited the agglutination reaction. The buffered antiserum was sterile-filtered and either stored in bulk or transferred in 2-ml amounts to 10- by 75-mm glass tubes that were stoppered and stored at 4°C.

Urine specific gravity measurements were made with a calibrated refractometer (National Instrument, Model 79029).

For preparation of laboratory serum standards blood was collected from healthy adults, allowed to clot, centrifuged for 10 min at 1100 *g*, decanted, and pooled. The pooled serum was tested by both latex tube test and radioimmunossay to insure the absence of detectable barbiturate, subdivided into 10-ml aliquots, frozen in serum bottles (Wheaton, Millville, N.J.), and stored at −15°C.

Procedures

For the latex tests, 2.0 ml of buffered antiserum was dispensed into 10- by 75-mm glass tubes. Specimen urine or control urine (0.5 ml), free of gross debris, was added, followed by 0.1 ml of coupled latex reagent. Where removal of gross debris was necessary, urines were first centrifuged for 10 min at 1100 *g* and then 0.5 ml of the clear supernatant fluid was removed for testing. The tubes were stoppered and the contents mixed by inverting twice or the unstoppered tubes were placed on a vortex mixer. The tubes were placed in a 37°C thermally regulated aluminum block (Lab-Line, Melrose Park, Ill., Model 2093) to a depth of approximately 1.5 cm from the bottom of the tube. After 2 h, the tubes were removed from the block and examined under fluorescent light. The results were noted as follows: 4+, large aggregates with some precipitation and clear surrounding fluid; 3+, large aggregates with clear surrounding fluid; 2+, agglutination with incomplete clearing of the surrounding fluid; 1+, small nonconvecting aggregates with turbid surrounding fluid; ½+, extremely fine nonconvecting particles with turbid surrounding fluid; and 0, a translucent fluid with no discernible agglutination (see Fig. 1).

Radioimmunoassays were performed with Abuscreen® radioimmunoassay for barbiturates according to the package directions. A gamma counter (Searle-Analytic Inc., Des Plaines, Ill., Model 1285) with 73% counting efficiency was used.

Results

Performance Characteristics

Agglutination Response—The following variables were examined to determine their effects upon barbiturate-latex agglutination-inhibition: dilution of the antiserum, specific gravity of the urine specimen, temperature of the reaction mixture, sample size, and type of barbiturate employed as the standard. These factors were examined by varying one parameter at a time and the following results were obtained.

As shown in Table 1, a more concentrated antiserum solution resulted in an increase in agglutination for the normal urine and a decrease in sensitivity to the barbiturate standards.

The degree of agglutination for urine samples was affected by the urine specific gravity (Table 2). At specific gravities of 1.005 to 1.030, agglutination of the negative control was 4+; at 1.040, it was 2+. As the specific gravity of urine, in which the standards were prepared, increased the amount of barbiturate required for inhibition decreased.

The degree of agglutination varied directly with the incubation temperature when the

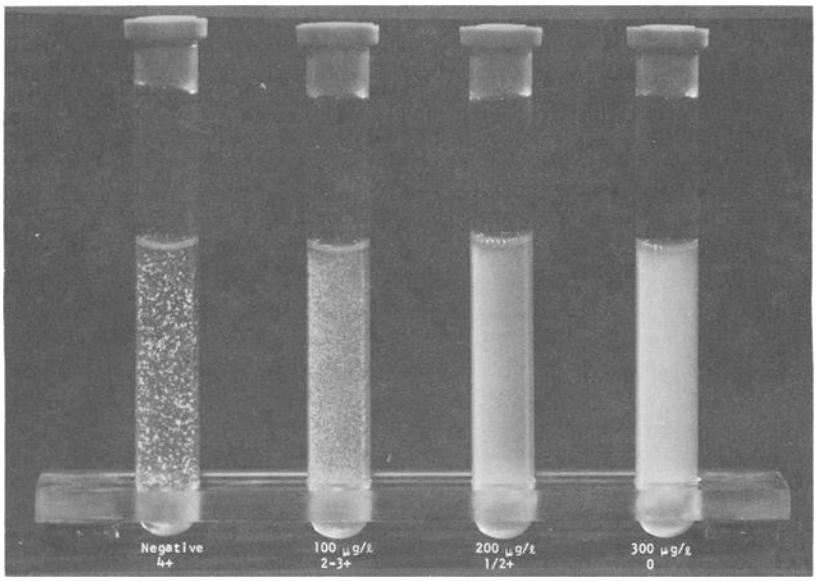

FIG. 1—*Typical agglutination pattern.*

TABLE 1—*Agglutination inhibition as a function of antiserum dilution.*

Secobarbital Concentration, µg/litre	Degree of Agglutination at the Indicated Dilution					
	1:100	1:95	1:90	1:85	1:80	1:67
Negative (barbiturate-free) urine	3-4	3-4	4	4	4	4
300	½	½-1	½-1	1	1	2
500	0	0	0	½	½	½-1

TABLE 2—*Agglutination as a function of urine specific gravity.*

Secobarbital Concentration, µg/litre	Specific Gravity				
	1.040	1.030	1.020	1.015	1.005
Negative (barbiturate-free) human urine	2	4	4	4	4
100	½	1-2	2	2	4
200	0	½	1	1	3
300	0	0	½	½-1	2
500	0	0	0	0	1
1000	0	0	0	0	½

time of incubation was maintained constant. When the block temperature was increased from 37 to 39°C an increase in agglutination of 1+ was observed; this represented a decrease in detectability of secobarbital from ~300 to ~500 μg/litre. When the block temperature was decreased from 37 to 35°C, an agglutination decrease of ½+ was obtained; this decrease in agglutination did not significantly alter detectability. Within the ±0.5°C range maintained by the thermally regulated blocks, no discernible differences were evident.

The effect of 3 to 20% variations in sample size was ascertained with the block temature at 37°C. An agglutination increase of 1+ was observed with a 0.400-ml sample, and this represented a decrease in detectability from ~300 to ~400 μg/litre. With a 0.600-ml sample a slight decrease in agglutination was evident, but this did not affect detectability. From 0.485 to 0.515 ml (±3%), which exceeds the allowable error of most pipets, no discernible differences in agglutination were noted.

The concentration of antiserum and antigen reagents was adjusted to give a 4+ agglutination with a negative urine control and no (0) agglutination with a 500-μg/litre secobarbital urine control. The affinity between four other barbiturates (amobarbital, pentobarbital, butabarbital, and phenobarbital) and the secobarbital-produced antibody was determined. Various concentrations of pentobarbital, butabarbital, amobarbital, and phenobarbital in urine were compared for their ability to inhibit agglutination with secobarbital standards prepared in the same urine pool. The results, shown in Table 3, indicate that complete inhibition was achieved with 500 μg/litre of secobarbital, compared with 2500 μg/litre for pentobarbital and butabarbital and 5000 μg/litre for amobarbital and phenobarbital. The order of relative activity was consistent with the radioimmunoassay data previously reported [3]. With the serum standards, complete inhibition was obtained at 100 μg/litre with secobarbital and pentobarbital and at 300 μg/litre with butabarbital, amobarbital, and phenobarbital. Since the latex test for secobarbital detects other barbiturates at differing rates, all subsequent results are expressed in terms of secobarbital equivalents (SE).

Stability—Antiserum and latex reagents were stored and examined at monthly intervals. The resultant data indicated that performance of the reagents was consistent for at least nine months at 5°C and at least three months at 25°C.

Clinical Testing

Background Values—The sensitivity of the latex agglutination-inhibition test for barbiturates can be varied by proper adjustment of the antiserum. However, for screening purposes a cutoff level of 400 μg/litre SE was chosen.

TABLE 3—*Agglutination inhibition as a function of the barbiturate and its concentration in urine.*

Concentration, μg/litre	Degree of Agglutination for the Indicated Barbiturate				
	Secobarbital	Pentobarbital	Butabarbital	Amobarbital	Phenobarbital
100	2	3-4	4	4	4
300	½	2	2	4	3
500	0	2	2	3	2
1000	0	1	1	2	½-1
2500	0	0	0	½-1	½-1
5000	0	0	0	0	0

To determine the potential for false positive results in individuals selected from a random population, urines were collected from 100 individuals and tested for the presence of barbiturate by the latex tube test. Ninety-five were negative for barbiturate (that is, less than 400 μg/litre SE), three were positive, and two were designated inconclusive. All specimens were also tested by radioimmunoassay: the two positive urines contained greater than 400 μg/litre SE; the third positive specimen contained greater than 200 μg/litre SE; and the two inconclusive specimens were negative at the 100 μg/litre SE level. However, one latex-inconclusive specimen which by radioimmunoassay contained 90 μg/litre SE had a specific gravity of 1.030. The other latex-inconclusive specimen which contained 40 μg/litre SE equivalents by radioimmunoassay had a specific gravity of 1.041. Consequently, a cutoff value of 400 μg/litre SE did not seem to present a significant problem in terms of false positive results.

Cross-Reactivity—Urines and sera from 13 subjects, who had taken orally one of 13 commonly prescribed drugs, were collected at timed intervals and examined to determine the effect of any of these drugs on the agglutination reaction.

All urine samples were negative (that is, showed 2+ to 4+ agglutination by the latex test) and complete agreement (100%) was observed with the radioimmunoassay (Tables 4 and 5). Decreased agglutination (2+) was obtained with urines having high specific gravities (1.030 and greater).

All serum samples from the same subjects were negative for barbiturate by the latex test and the absence of barbiturates was also confirmed by the radioimmunoassay (Tables 4 and 5). The degree of agglutination for barbiturate-free serum samples was, on the average, less than that noted for the barbiturate-negative urines from the same subjects.

Subject Studies—The barbiturate-latex test was used to monitor barbiturate levels in 110 urine and 99 serum specimens from eleven subjects who had taken orally one of the following: secobarbital, amobarbital, pentobarbital, butabarbital, or phenobarbital. The excretion profile data are summarized in Tables 6 and 7.

TABLE 4—*Cross-reactivity data.*

Generic Name	Trade Name	Amount Administered	Latex Result	
			Urine	Serum
Chlordiazepoxide	Librium	10 mg	neg. (2-4+)[a]	neg. (2-4+)[b]
Diazepam	Valium	5 mg	neg. (2-4+)	neg. (3+)
Chlorpromazine	Thorazine	10 mg	neg. (2-3+)	neg. (3+)
Caffeine	...	260 mg	neg. (3-4+)	neg. (2-3+)
Glutethimide	Doriden	500 mg	neg. (3-4+)	neg. (2+)
Oxyphenbutazone	Tandearil	100 mg	neg. (2-4+)	neg. 2-3+)
Phenylbutazone	Butazolidin	100 mg	neg. (2-4+)	neg. (3+)
Promethazine	Phenergan	25 mg	neg. (2-4+)	neg. (2-3+)
Diphenylhydantoin	Dilantin	100 mg	neg. (2-4+)	neg. (3+)
Trifluoperazine	Stelazine	1 mg	neg. (3-4+)	neg. (3+)
Methazualone	Quāālude	300 mg	neg. (2-4+)	neg. (2+)
Chloroquine	Aralen Phosphate	500 mg	neg. (2-4+)	neg. (2-3+)
Methyprylon	Noludar	300 mg	neg. (4+)	neg. (2+)

[a] Range of values for all specimens collected from 0 to 120 h.

[b] Range of values for all specimens collected from 0 to 72 h.

TABLE 5—*Comparison of radioimmunoassay (RIA) and latex tube test data for specimens containing commonly prescribed drugs.*

Specimen Type	Specimens, *n*	RIA Barbiturate Concentration, μg/litre SE	Latex Tube Test					
			Positive		Negative		Inconclusive	
			n	%	*n*	%	*n*	%
Negative urines	130	<12.5	0	0	130	100	0	0
Negative sera	117	<12.5	0	0	117	100	0	0

The data for urine specimens showed that secobarbital, pentobarbital, butabarbital, and phenobarbital were detected for as long as 96 to 120 h (maximum time period tested) after drug administration. Amobarbital was detected only at the 4- to 8-h and 12- to 24-h intervals. The latex test detected barbiturates excreted in urine at lower concentrations than anticipated from the response patterns obtained with the individual in-vitro standards. Phenobarbital, in particular, based on the corresponding radioimmunoassay data, was detected at levels as low as 150 μg/litre SE. In four instances (pentobarbital 1 to 4 h and 8 to 12 h; butabarbital 1 to 4 h and 4 to 8 h) where the radioimmunoassay indicated greater than 400 μg/litre SE and the latex test gave inconclusive results, the specimens had low specific gravities (1.005 to 1.007).

The data for serum specimens showed that secobarbital, pentobarbital, butabarbital, and phenobarbital were detected for as long as 72 h (maximum time period tested) after drug administration. Amobarbital was not detected in serum.

A comparison of the radioimmunoassay with the latex tube test as a function of specimen concentration is indicated in Table 8. In urine, barbiturates were detected in 100% of the specimens with >1000 μg/litre SE, 62.5% of the specimens with 500 to 1000 μg/litre SE, 87.5% of the specimens with 250 to 499 μg/litre SE, 50% of the specimens with 100 to 249 μg/litre SE, and 0% of the specimens with 0 to 99 μg/litre SE. In serum, barbiturates were detected in 100% of the specimens with >1000 or 500 to 1000 μg/litre SE, 68.8% of the specimens with 250 to 499 μg/litre SE, 85.7% of the specimens with 100 to 249 μg/litre SE, and 0% of the specimens with 0 to 99 μg/litre SE.

Reproducibility—Ten urine specimens from six subjects were tested in quintuplicate on three separate days. The specimens represented a full range of barbiturate concentration: negative, low, medium, and high levels. As indicated in Table 9 there was no significant within-day or between-day variation in the agglutination results for specimens containing ≤150 μg/litre SE or ≥543 μg/litre SE. The minimum perceivable agglutination change resulted in some between-day variation for specimens containing ~300 μg/litre SE.

Discussion

The latex tube test detected a variety of barbiturates in urine and in serum. Some barbiturates were detected at lower levels than anticipated from the response patterns obtained with the individual in-vitro standards (Table 3). Phenobarbital was detected at 150 μg/litre SE in urine instead of the 5000-μg/litre SE level observed with in-vitro standards. Conversely, amobarbital was not detected at 300 to 360 μg/litre in serum specimens, whereas complete inhibition was obtained with a 300-μg/litre laboratory serum standard.

TABLE 6—*Urine test results as a function of time following administration of selected barbiturates; barbiturate concentrations in units of μg/litre SE.*

	Secobarbital, 100 mg		Amobarbital, 65 mg		Pentobarbital, 100 mg		Butabarbital, 50 mg		Phenobarbital, 64 mg	
Time, h	Latex[a]	RIA	Latex	RIA	Latex	RIA	Latex	RIA	Latex	RIA
0	neg.	0	neg.	3	neg.	0	neg.	3	neg.	0
0-1	pos.	>1000	neg.	60	inc.	120	pos.	395	neg.	0
1-4	pos.	>1000	neg.	180	inc.	425	inc.	>500	neg.	95
4-8	pos.	>1000	pos.	400	pos.	660	inc.	>500	pos.	195
8-12	pos.	>1000	neg.	150	inc.	550	pos.	>500	inc.	135
12-24	pos.	>1000	pos.	450	pos.	830	pos.	340	pos.	180
24-48	pos.	>1000	inc.	150	pos.	>1000	pos.	430	pos.	175
48-72	pos.	>1000	neg.	95	pos.	>1000	pos.	500	pos.	255
72-96	pos.	>1000	inc.	90	pos.	>1000	inc.	195	pos.	175
96-120	pos.	390	neg.	30	pos.	510	pos.	220	pos.	150

[a] Latex agglutination-inhibition test results were recorded at 120 min as positive (translucent endpoint), negative (2 to 4+ bold agglutination), or inconclusive (cloudy, nontranslucent, or fine ½ to 1+ agglutination).

TABLE 7—*Serum test results as a function of time following administration of selected barbiturates; barbiturate concentrations in units of μg/litre SE.*

	Secobarbital, 100 mg		Amobarbital, 65 mg		Pentobarbital, 100 mg		Butabarbital, 50 mg		Phenobarbital, 64 mg	
Time, h	Latex[a]	RIA	Latex	RIA	Latex	RIA	Latex	RIA	Latex	RIA
0	neg.	< 12.5	neg.	0	neg.	0	neg.	0	neg.	0
1	pos.	>1000	neg.	37	pos.	220	pos.	>500	inc.	30
2	pos.	>1000	inc.	300	pos.	305	pos.	>500	pos.	220
4	pos.	>1000	inc.	360	pos.	315	pos.	>500	pos.	385
8	pos.	>1000	inc.	345	pos.	308	pos.	>500	pos.	330
12	pos.	>1000	inc.	320	pos.	321	pos.	>500	pos.	340
24	pos.	855	inc.	260	pos.	239	pos.	>500	pos.	325
48	pos.	315	neg.	115	pos.	145	pos.	>500	pos.	265
72	pos.	171	neg.	15	pos.	355	pos.	>500	pos.	245

[a]Latex agglutination-inhibition test results were recorded at 120 min as positive (translucent endpoint), negative (2 to 4+ bold agglutination), or inconclusive (cloudy, nontranslucent, or fine ½ to 1+ agglutination).

TABLE 8—*Latex tube test data as a function of specimen concentration by RIA.*

Specimens, *n*	RIA Barbiturate Concentration, μg/litre SE	Latex Tube Test					
		Positive		Negative		Inconclusive	
		n	%	*n*	%	*n*	%
		Urine					
11	>1000	11	100	0	0	0	0
8	500-1000	5	62.5	0	0	3	37.5
8	250-499	7	87.5	0	0	1	12.5
12	100-249	6	50	2	16.7	4	33.3
11	0-99	0	0	10	90.1	1	0.9
		Serum					
5	>1000	5	100	0	0	0	0
9	500-1000	9	100	0	0	0	0
16	250-499	11	68.8	0	0	5	31.2
7	100-249	6	85.7	1	14.3	0	0
8	0-99	0	0	7	87.5	1	12.5

At present, the differences in the minimum concentration that can be detected in laboratory standards versus subject specimens cannot be adequately explained. However, detection of barbiturates in serum standards at lower concentrations than in urine standards might be explained on the basis of protein suppression of agglutination. This phenomenon has been observed with a morphine latex test and was attributed to the effect of serum proteins in inhibiting particle agglutination [*6*].

Although sensitivity towards laboratory standards could be increased by decreasing the amount of antiserum in the formulation, it would not be advantageous for drug screening, specifically for the detection of the presence of barbiturates. Normal variation in specimen urine specific gravity could decrease the degree of agglutination between positives and negatives, which would affect readability and increase the possibility of false positive results.

TABLE 9—*Reproducibility study.*

Barbiturate Concentration, μg/litre SE	Degree of Agglutination on Indicated Day[a]						
	1(M)	1(A)	2(A)	3(M)	3(A)	Expected Result	ER/TO,[b] %
0[c]	4+(5/5)	4+(5/5)	4+(5/5)	4+(5/5)	4+(5/5)	neg.	100
9	3+(5/5)	3+(5/5)	3+(5/5)	4+(5/5)	4+(5/5)	neg.	100
146	2+(5/5)	2+(5/5)	2+(5/5)	1+(5/5)	1+(5/5)	neg.	100
185	3+(5/5)	3+(5/5)	3+(5/5)	3+(5/5)	3+(5/5)	neg.	100
284	±(5/5)	±(5/5)	±(5/5)	−(0/5)	−(0/5)	inc.	60
329	±(5/5)	±(5/5)	±(5/5)	−(0/5)	−(0/5)	inc.	60
558	−(5/5)	−(5/5)	−(5/5)	−(5/5)	−(5/5)	pos.	100
543	−(5/5)	−(5/5)	−(5/5)	−(5/5)	−(5/5)	pos.	100
2700	−(5/5)	−(5/5)	−(5/5)	−(5/5)	−(5/5)	pos.	100
>5000	−(5/5)	−(5/5)	−(5/5)	−(5/5)	−(5/5)	pos.	100

[a] M = morning; A = afternoon; + = agglutination or a negative result; ± = an inconclusive result; − = no agglutination or a positive result.

[b] Ratio of the expected results (ER) to the total observations (TO).

[c] Average of duplicate radioimmunoassays.

A positive latex test result was highly indicative of the presence of barbiturates of the type studied in this report. Furthermore, without identifying the specific compound (secobarbital, amobarbital, pentobarbital, butabarbital, or phenobarbital), barbiturates were detected by latex agglutination based on quantitation by radioimmunoassay 100% of the time at a concentration of >1000 μg/litre SE in urine specimens, 62% of the time at a concentration of 500 to 1000 μg/litre SE in urine specimens, and 100% of the time at a concentration of >500 μg/litre SE in serum specimens. A negative result, except for amobarbital, was equally indicative of the absence of barbiturates.

Positive and doubtful test values should be confirmed by another technique. As with any immunoassay, it is necessary to use a nonimmunologic confirmatory test to establish the presence of a drug with certainty.

In conclusion, the indirect latex test is simple, rapid, and sensitive. The equipment necessary to perform the test includes a 37°C heated block and quantitative pipets. Except for removal of gross debris pretreatment of the sample is not required for the tube test. The specificity and sensitivity of the latex tube test appear to be intermediate between radioimmunoassay and thin-layer chromatography [7].

Summary

A latex-agglutination inhibition test was developed and evaluated for the detection of barbiturates, primarily in urine, but it has applicability to serum. The 2-h test was performed with samples free of gross debris in a 37°C heat block and the reaction between the barbiturate antibody and the barbiturate-latex, at pH 7.3, was inhibited by urines containing secobarbital, amobarbital, pentobarbital, butabarbital, or phenobarbital. For laboratory standards the test was particularly sensitive to secobarbital (300 μg/litre) and relatively insensitive to amobarbital (5000 μg/litre). In clinical specimens some barbiturates were detected at levels as low as 150 μg/litre. The test was specific, and negative endpoints were frequently noted in as little as 30 min. The effects of variables (antiserum dilution, urine specific gravity, heat block temperature, sample size, and type of barbiturate) on the degree of agglutination were also determined.

Acknowledgments

We gratefully acknowledge the cooperation of Dr. R. Pocelinko under whose supervision clinical samples were obtained, Mr. R. Kucan for supplying other essential samples, and Annie Harris for assisting in the reproducibility study.

References

[1] Spector, S. and Flynn, E. J., "Barbiturates: Radioimmunoassay," *Science,* Vol. 174, Dec. 1971, pp. 1036–1038.

[2] Flynn, J. and Spector, S., "Determination of Barbiturate Derivatives by Radioimmunoassay," *Journal of Pharmacology and Experimental Therapeutics,* Vol. 181, No. 3, March 1972, pp. 547–554.

[3] Cleeland, R., Davis, R., Heveran, J., and Grunberg, E., "A Simple, Rapid ^{125}I Radioimmunoassay for the Detection of Barbiturates in Biological Fluids," *Journal of Forensic Sciences,* Vol. 20, No. 1, Jan. 1975, pp. 45–57.

[4] Schneider, R., Bastiani, R., Leute, R., et al, "Use of Enzyme and Spin Labeling in Homogeneous Immunochemical Detection Methods," in *Immunoassays for Drugs Subject to Abuse,* S. Mule, I. Sunshine, M. Brande, and R. Willete, Eds., CRC Press, Cleveland, Ohio, 1974, pp. 45–72.

[5] Alexander, G. I., "Direct Use of Ion-Exchange Papers in Hemagglutination-Inhibition Tests for Drug Abuse," *Clinical Chemistry,* Vol. 22, July 1976, pp. 1105–1106.

[6] Ross, R., Horwitz, C. A., Hager, H., Usategui, M., Burke, M. D., and Ward, C. J., "Preliminary Evaluation of a Latex Agglutination Tube Test for Morphine," *Clinical Chemistry,* Vol. 21, Jan. 1975, pp. 139-143.

[7] Roerig, D. L., Lewand, D. L., Mueller, M. A., and Wang, R. I. H., "Comparison of Radioimmunoassay with Thin-Layer Chromatographic and Gas-Liquid Chromatographic Methods of Barbiturate Detection in Urine," *Clinical Chemistry*, Vol. 21, May 1975, pp. 672-675.

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