David A. Armbruster;[1] *Robert H. Schwarzhoff;*[1]
Barbara L. Pierce;[1] *and Edward C. Hubster*[1]

Method Comparison of EMIT II and OnLine with RIA for Drug Screening

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ABSTRACT: The newest formulation of the EMIT assay for drugs of abuse, EMIT II, and a new immunoassay, OnLine, using the kinetic interaction of microparticles in solution methodology, were evaluated for marijuana, cocaine, opiates, barbiturates, and phencyclidine. Both types of immunoassays were performed on an Hitachi 717 analyzer. Calibration curves, the degree of separation between negative and cutoff calibrators, precision, probability of carryover from positive to negative samples, and overall ease and speed of analysis were evaluated. EMIT II and OnLine were compared with RIA tests for the five drugs to determine each assay's ability to detect samples which confirm positive by GC/MS. The RIA and OnLine marijuana tests detected >99% of confirmed positive samples while EMIT II detected about 90%. All three immunoassays performed equivalently for cocaine and opiates, each assay detecting at least 98% of positives. Barbiturates showed the greatest disparity with OnLine detecting 96%, EMIT II 85%, and RIA 79% of confirmed positive samples. Too few phencyclidine positive samples were detected for a method comparison study. The fully automated EMIT II and OnLine assays are preferable for a variety of reasons to our laboratory's current semi-automated RIA tests for large volume urine testing. The immunoassays offer comparable performance for some drugs but not for others.

KEYWORDS: toxicology, drug screening, chemical analysis, RIA, EMIT

Immunoassay screening tests are used to eliminate negative urine samples, usually the overwhelming majority, from further consideration so that full attention can be focused on the presumptive positives. These samples are typically confirmed positive using gas chromatography/mass spectrometry (GC/MS), a sophisticated and very specific methodology. The significance of the immunoassay screen cannot be underplayed because it is the quality of this test that ultimately determines how effective a drug testing program is in detecting and deterring the use of abused drugs.

Recently, Syva released a new formulation (EMIT II) of its widely used enzyme immunoassay and Roche introduced a new assay, OnLine, using a unique methodology based on the agglutination of microparticles. We evaluated the operating characteristics

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[1]Chief, Quality Assurance, Air Force Drug Testing Laboratory.

[2]Technical Director; Chemist, Analytical Sciences Branch; and Chief, RIA Section, respectively, Brooks AFB, TX.

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of both of these assays, as performed on an Hitachi 717 analyzer, and compared them to each other and to our routine RIA procedures for marijuana, cocaine, opiates, phencyclidine, and barbiturates. Over 50 000 routine urine samples were screened using all three immunoassays for these drugs and samples testing positive by one or more of the tests were submitted for confirmation testing by GC/MS. The number of confirmed positives detected by each immunoassay were calculated. This study was undertaken to determine the feasibility of replacing our standard RIA screening tests with nonradioisotopic assays. The objective was to gain a practical appreciation of the strengths and weaknesses of the nonradioisotopic tests vis-a-vis RIA and to determine whether the ability to detect confirmable positive urines would be increased, decreased, or remain about the same by switching to a different screening methodology.

Methods

Screening

Batches of 200 urine samples, submitted to our laboratory from Air Force and Army units, were tested using routine RIA procedures in accordance with the Department of Defense's (DOD) Drug Testing Program. Each batch was assayed for marijuana, cocaine, and a third "pulse" drug, either opiates, phencyclidine, or barbiturates, the pulse drug being rotated on a monthly basis. After the samples were released from the forensic chain-of-custody, they were analyzed for the same drugs on an Hitachi 717 Analyzer using EMIT II and OnLine reagents. Two 717's were used, one dedicated to EMIT II reagents and the other to OnLine reagents, to prevent any potential adverse effects of using both reagent systems on the same analyzer. Presumptive positive samples, detected either through routine forensic testing or by either EMIT II or OnLine, were saved and submitted for GC/MS confirmation.

RIA

Roche Abuscreen Kits (Roche Diagnostic Systems, Nutley, NJ) were used as per the manufacturer's directions. Batches of 200 samples, consisting of 190 actual samples plus ten internal blind controls (five negative, five positive), were processed in conjunction with 40 calibrators and open controls (ten cutoff calibrators plus negative, low, cutoff, and high controls). Batches were processed using Micromedic Automatic Pipetting Stations (ICN Micromedic Systems, Huntsville, AL) and Micromedic Apex ten-well gamma counters. The DOD Drug Testing Program requires that screening be performed using RIA tests and other immunoassays are not presently authorized. These RIA procedures have been extensively validated by the DOD program and our laboratory.

EMIT II

EMIT II (Syva Company, San Jose, CA) tests were performed on an Hitachi 717 Analyzer (Boehringer Mannheim Corporation, Indianapolis, IN) as per the manufacturer's directions using Syva instrument parameters for the 717. The urine samples, contained in the 12 × 75-mm glass test tubes used for RIA testing, were removed from the RIA racks and loaded into 60-place Hitachi sample discs configured to accommodate the tubes rather than the usual plastic sample cups. While the samples were released from the forensic chain-of-custody, they remained in the original aliquot tubes and were not re-aliquoted. The sample discs were transferred from one 717 after analysis was completed to the other 717 for analysis by the other immunoassay being evaluated.

OnLine

Roche OnLine tests were performed on an Hitachi 717 analyzer as per the manufacturer's directions using Roche instrument parameters for the 717. The parameter settings supplied by the manufacturer were not necessarily optimized for the DOD cutoffs which vary from the National Institute on Drug Abuse (NIDA) mandated values for marijuana and cocaine. Analysis was performed as described previously for EMIT II testing. The order of analysis (EMIT II followed by OnLine or OnLine followed by EMIT II) varied from day to day.

GC/MS Confirmation

Presumptive positives for marijuana, cocaine, opiates, and phencyclidine were analyzed by GC/MS using our standard, validated assays performed on Hewlett-Packard 5890 GC/5970 MSD systems (Hewlett-Packard, Atlanta, GA). Because of the small number of barbiturate presumptive positives typically detected, those samples were sent for confirmation to Northwest Toxicology, Inc., Salt Lake City, UT, as per our usual procedure.

Calibration Study

Absorbance rate change data (EMIT II) and absorbance change data (OnLine) for the negative, cutoff, and high calibrators were collected for 20 days. The means, SDs, and CVs for the calibrators were calculated and average calibration curves drawn. As an approximation of the degree of separation realized between the negative and cutoff calibrators, the slopes of the calibration curves between these two points were calculated. OnLine calibration kits contain five levels of calibrator and the assays can be performed in a quantitative mode if all calibrators are used. We chose to use only three calibrators and to use the OnLine kits in the semiquantitative, positive or negative mode typically used by drug testing laboratories.

Precision

Day-to-day precision was determined by calculating the mean, SD, and CV of calibrator values collected over 20 days.

Carryover

Carryover from one sample to another on the Hitachi 717 was determined by assaying high concentration (H) samples and low concentration (L) samples in the following sequence [*1*].

$$L_1L_2L_3H_1H_2L_4H_3H_4L_5L_6L_7L_8H_5H_6L_9H_7H_8L_{10}H_9H_{10}L_{11}$$

Each assay's high calibrator was used for the H samples and the low calibrators were used for the L samples to simulate a moderate carryover scenario. Percent carryover is calculated as:

$$\%\ \text{CARRYOVER} = \frac{(L_4 + L_5 + L_9 + L_{10} + L_{10})/5 - (L_2 + L_3 + L_6 + L_7 + L_8)/5}{(L_2 + L_3 + L_6 + L_7L_8)/5} \times 100$$

Method Comparison

Samples that screened positive by any immunoassay and that were confirmed by GC/MS were defined as true positives. Samples were tested between January and May, 1992. Some screening and confirmation cutoff values for the DOD program changed as of 1 Jan 92, and the appropriate cutoff values are listed in Table 1. For both screen and confirm data, the DOD cutoff values were used to designate negative and positive samples. Note that some values are lower than NIDA cutoffs.

Results and Discussion

Assay Performance

The EMIT methodology is well established and widely used and need not be described here. EMIT II differs from earlier formulations in that it uses new polyclonal antibodies with improved specificities and a new formulation of drug-glucose-6-phosphase dehydrogenase conjugates. The purpose of these changes are to realize improved performance at the cutoff level. The EMIT II reagents are available in large (100 and 500 mL) kits which make them convenient for high-throughput analyzers in high volume laboratories. The 500 mL kit, for example, is sufficient for about 3800 tests on the Hitachi 717. EMIT II reagents come with lot-specific information for five common analyzers which list the expected absorbance rate separations between the negative and cutoff and the cutoff and high calibrators. EMIT II uses a separate calibration kit for marijuana and a combined calibration kit for amphetamines, opiates, cocaine, phencyclidine and barbiturates. We have reported elsewhere on our comparison of EMIT II with EMIT 700 reagents [See Ref. 2]. We found that, in general, the operational characteristics of EMIT II offer advantages over EMIT 700, particularly for the marijuana assay.

OnLine represents a new immunoassay methodology—the kinetic interaction of microparticles in solution, or KIMS. The KIMS methodology is illustrated in Fig. 1. In any typical immunoassay, the drug of interest is conjugated to a "tag," or analytical signal producing entity, for example, a radioisotope, an enzyme, or a fluorescent molecule. In KIMS, the conjugate reagent consists of drug bound to microparticles. The microparticle conjugate in solution minimally blocks light transmission through a cuvette. When antibody to the drug is added to the conjugate in solution, lattice formation takes place. The

TABLE 1—*DOD cutoff values in ng/mL for screening and confirmation as of 1 January, 1992.*[a]

	Drug	Cutoff
Screening	Marijuana	50
	Cocaine	150
	Opiates	300
	Barbiturates	200
	Phencyclidine	25
Confirmation	Marijuana	15
	Cocaine	100
	Opiates	
	Codeine	300
	Morphine	300
	Barbiturates	200
	Phencyclidine	25

[a]The DOD screening cutoffs for marijuana and cocaine are one half of the NIDA cutoffs and that the cocaine confirmation cutoff is 50 ng/mL lower than the NIDA value.

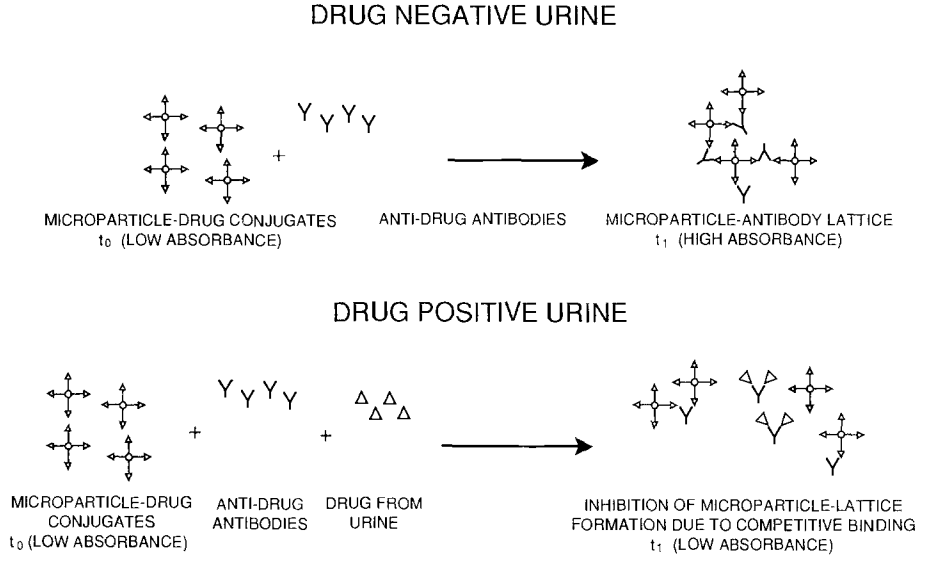


FIG. 1—*The kinetic interaction of microparticles in solution (KIMS) methodology in the case of a negative urine and for a urine containing the drug or drug metabolite(s) of interest.*

resulting microparticle lattice effectively blocks light transmission and increases absorbance. Addition of a drug-free urine sample to the OnLine conjugate and antibody reagents will not interfere with microparticle lattice formation and absorbance will increase over a given time period. Unlike EMIT which measures an enzymatic absorbance rate change, OnLine employs an "end point" measurement, the difference in absorbance between an initial and final reading. If a urine sample containing the drug of interest is mixed with the OnLine reagents, free drug in the sample will compete with conjugate for antibody binding sites and the degree or lattice formation is inhibited proportional to urine sample drug concentration. Final absorbance after a given reaction time decreases with increasing urine sample drug concentration. Use of drug calibrators at various concentrations allows a curvilinear calibration curve to be constructed. OnLine calibration packs contain five calibrators. All five calibrators may be used to provide semiquantitative results or the negative, cutoff, and a high calibrator may be used to provide simple "positive" or "negative" results. We used OnLine in the positive or negative mode.

Data presentation for the EMIT and OnLine assays varies with the analyzer used. The Hitachi analyzer measures the absorbance rate change for the EMIT assays or the absorbance change for the OnLine assays between set time points for each calibrator, control, or sample. If properly programmed, the raw absorbance data can be printed for either assay but typically the data is converted to "machine numbers." For example, the absorbance change value of the cutoff calibrator for any assay is calculated and is set to a machine value of "0." A negative sample will produce a negative machine number, for example, −20 (EMIT) or −300 (OnLine), because the negative sample produces an absorbance change that is so many absorbance units below that of the cutoff calibrator. Similarly, a positive sample will result in a machine number equal to "0" or a positive value, indicating that the absorbance change is equal to that of the cutoff calibrator or greater than it by so many absorbance units. Most laboratories use machine numbers to record calibrator, control, and sample results because these are the values routinely printed out. We used actual absorbance change data to construct calibration curves and make some precision calibrations but otherwise used the converted machine numbers.

In terms of time and effort required for analysis, both EMIT II and OnLine are preferable to our current RIA procedures. RIA requires several manual steps by the technician (loading aliquots on the pipetting station, incubation for half an hour, centrifugation, decanting, gamma counting, etc.). Analysis is basically sequential, that is, in order to test for three drugs, each assay must be set up one at a time. While one technician may perform all of the assays, it is not unusual for two or even three technicians to perform analyses, necessitating transfer of custody, as reflected on the aliquot chain-of-custody form. Daily calibration and set-up of the pipetting stations requires considerable time.

By contrast, preventive maintenance and daily set-up time of an Hitachi 717 for either EMIT II or OnLine requires only about 15 minutes. Both nonradio-isotopic assays were calibrated at the start of a shift and the analyzer was programmed to repeat analysis of the calibrators and open controls at predetermined intervals. Once samples are loaded on the 717, technician interaction was minimal. In fact, the only major manual operation is loading and unloading the 60 place sample disc containing the aliquots. The analyzer can perform three or more drug tests on each aliquot simultaneously, obviating the need to transfer custody of aliquots from one technician to another. The RIA procedures require about 2 and one-half hours to test a 200 aliquot batch for three drugs. EMIT II and OnLine can perform the same analysis on a 717 in about 1 and one-half hours. While bar coding is not currently used in our laboratory, it is projected in the near future and the Hitachi 717 is barcode capable. The use of barcodes would add another degree of certainty to the process and serve to expedite result reporting and review. In terms of ease of operation, analysis time, and overall efficiency, either EMIT II or OnLine as performed on an Hitachi 717-type analyzer is preferable to RIA procedures.

EMIT II and OnLine require about an equal amount of time to test the same number of samples for a given number of drugs on the Hitachi 717. Reconstitution of the OnLine reagents consists of simply mixing two bottles together and was found to be quicker and easier than preparation of EMIT II kits. On the other hand, EMIT II reagents are stable for 12 weeks while OnLine kits expire four weeks after reconstitution.

Calibration

Average calibration curves for EMIT II and OnLine assays are illustrated in Figs. 2 and 3. The EMIT II curves are very similar to those obtained with previous EMIT formulations except for marijuana (see Ref *2* for details). The marijuana assay calibration curve is much steeper than the typical EMIT assay calibration curve, suggesting enhanced ability of the EMIT II test to distinguish marijuana positives from negatives. Day-to-day CVs (n = 20) for the negative, cutoff, and high calibrators ranged from 0.8 to 5.6%. These values suggest that calibration curves remain reasonably stable for about three weeks.

OnLine calibration curves are also curvilinear but, due to the inverse relationship between absorbance change and analyte concentration, follow a negative slope. All OnLine calibration curves generally resemble each other and simply differ in how shallow or steep is the curve. Day-to-day CVs (n = 20) for the negative, cutoff, and high calibrators ranged from 2.8-8.0%. The CVs were generally higher than for the EMIT II curves, suggesting more variability in calibration from one day to another. Both manufacturers call for recalibration if a reagent is changed or if control values are unacceptable. The common practice observed in the field of calibrating daily or at the start of a new shift is probably prudent for optimal use of either EMIT II or OnLine.

One means of comparing immunoassay calibration curves is to calculate the slope of the curve between the negative and cutoff calibrators. EMIT II, OnLine, and RIA calibration curves are all curvilinear, so the slope, which is a linear relationship, can only be an approximation of the amount of change in analytical signal per change in drug

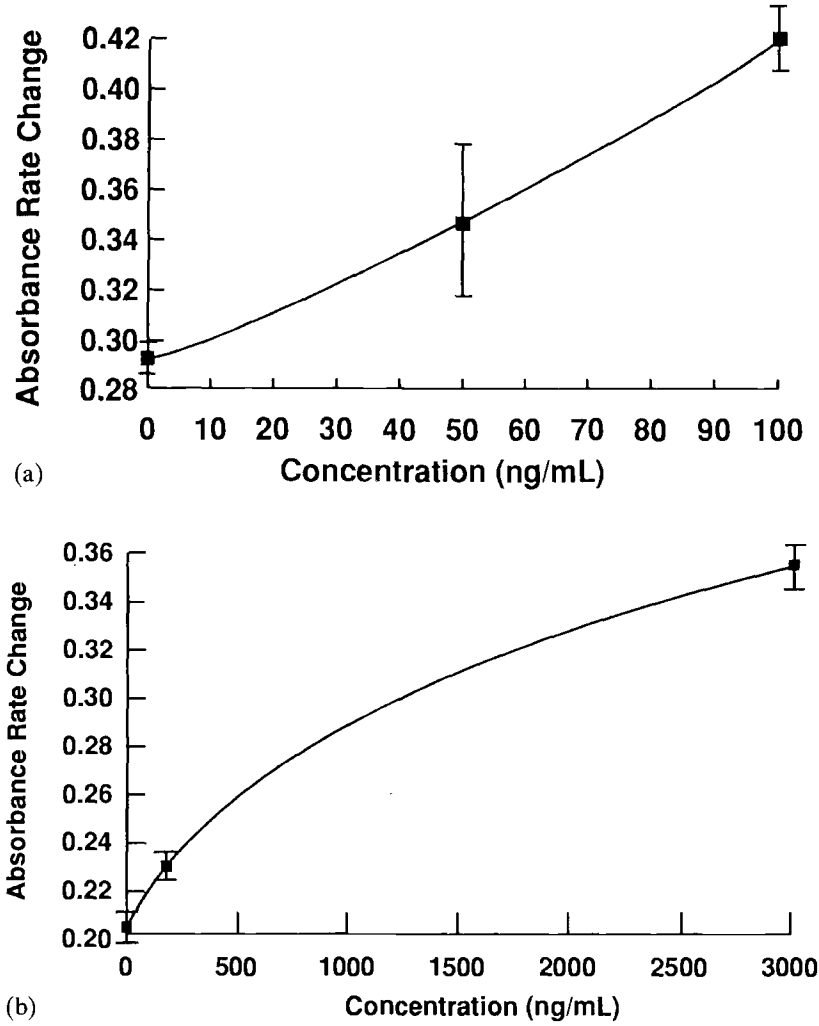
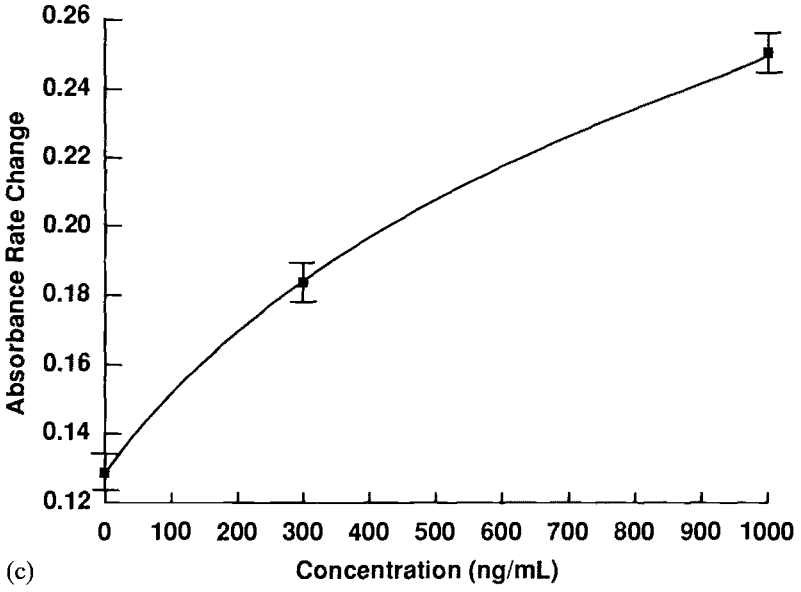


FIG. 2—*EMIT II calibration curves based on averaged daily data (n = 20). Error bars represent ±2 SD. (a) THC; (b) cocaine; (c) opiate; (d) barbiturates; (e) PCP.*

concentration over a stated range, but it is a convenient way of estimating assay "sensitivity." Sensitivity is often used synonymously for lower limit of detection but is really more correctly defined as the change in analytical response for a change in analyte concentration, as used here [*3,4*]. For the purposes of drug testing, the greater the sensitivity, estimated by the slope, the better the ability of an assay to distinguish a negative sample (one below the cutoff value) from a positive (one at or exceeding the cutoff). Table 2 lists the slopes for the EMIT II, OnLine, and RIA calibration curves. In all cases, the RIA slopes are considerably greater than EMIT II and OnLine slopes, and the OnLine slopes are larger than the EMIT II slopes. All phencyclidine assays exhibit considerably greater slopes than those of the other assays using the same methodology. After phencyclidine, all of the marijuana tests exhibit the next greatest slopes for the assays using the same methodology. The method comparison data presented here (see below) suggest that the degree of separation between an assay's negative and cutoff



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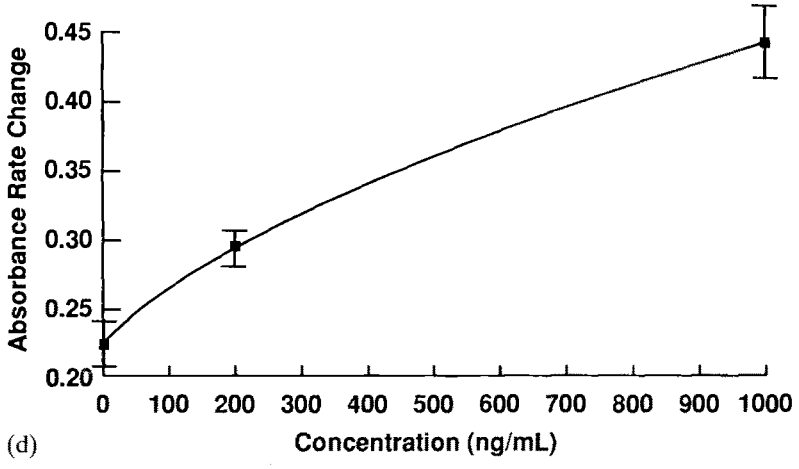


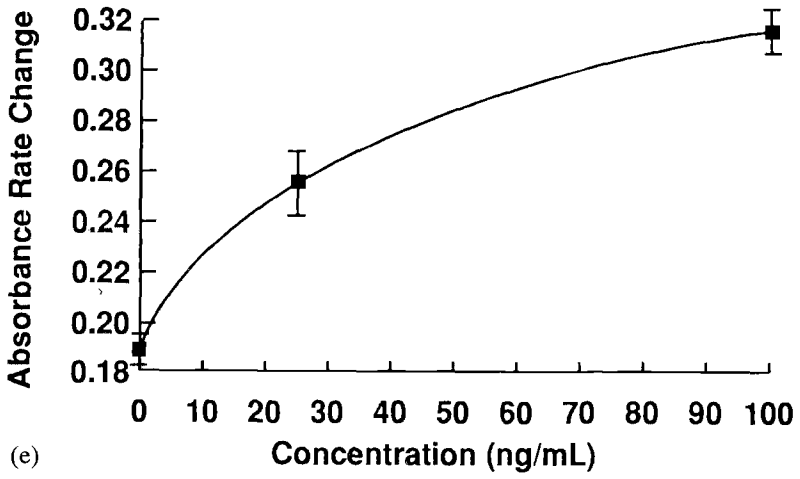
(d)

FIG. 2—*Continued.*

calibrators, estimated by slope, may be useful to predict an assay's ability to detect positive samples in some cases. In other instances, for example, the barbiturate assays studied here, a large separation between the negative and cutoff calibrators is not the only factor impacting an assay's effectiveness.

Precision

Day-to-day precision data for the calibrators for both types of assays are listed in Table 3. In most cases, CVs for both EMIT II assays are less than about 5% and for OnLine, less than 7%. In general, EMIT II assays appear to exhibit better precision than the OnLine assays. In comparison, within-run CV's for our current RIA kit calibrators and controls range from 1.1 to 3.8%. Meaningful day-to-day CVs for the RIA kits are



(e)

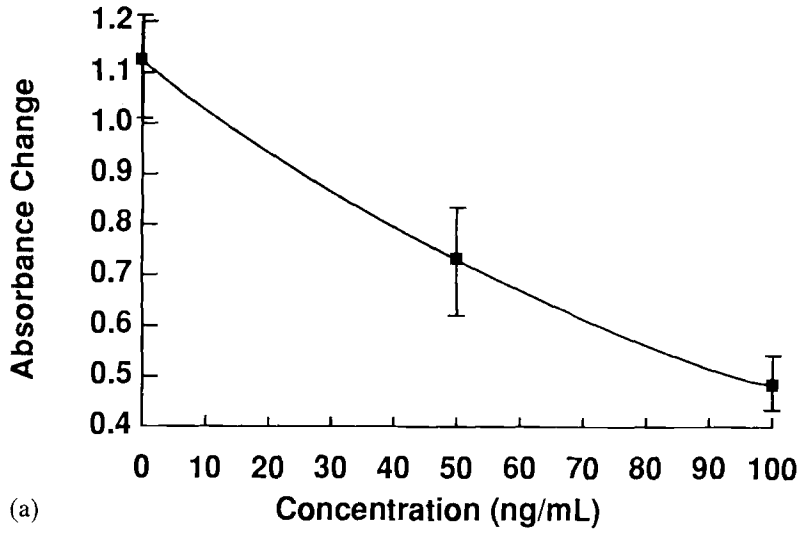
FIG. 2—*Continued.*

not available because the natural decay of the radioisotopic tag make these calculations using raw counts inappropriate. Precision of the nonradioisotopic assays might be improved by extending the incubation times, with the concomitant loss of speed of analysis. Typically, raw absorbance data is not used to monitor the precision of calibrators or controls. Instead the "machine numbers" generated by the analyzer from the absorbance readings are recorded. We found day-to-day precision to be higher when this data was used but CVs for both assays were still usually <10%.

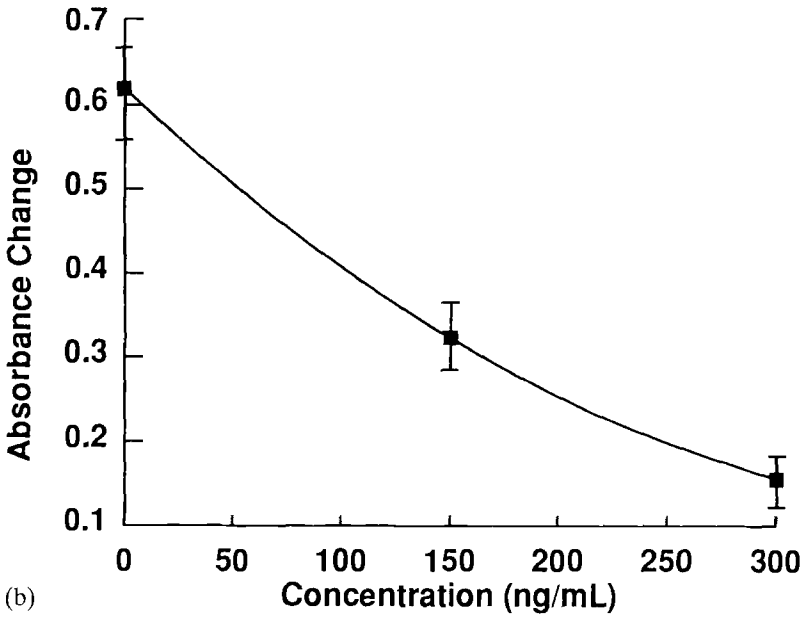
Carryover

Carryover of positive to negative samples following them is an occasional problem with the current RIA tests used in our laboratory, particularly with cocaine. Carryover is attributable to the limitations of the automatic pipetting systems used and to the extremely elevated drug concentration sometimes encountered. As all presumptive positives from the initial screening are rescreened using new aliquots which are manually pipetted, false screen positives due to carryover are eliminated. It was desirable to determine whether carryover is a significant problem with EMIT II or OnLine on the Hitachi analyzer, recognizing that carryover is more of an instrument than a reagent problem.

The results of the carryover study are given in Table 4. The approach used is described in the Methods Section. To simulate a moderate carryover scenario, the high calibrators from both assays were used for "high" samples and the low calibrators for "low" samples. As the negative calibrators for both assays yield negative readings, carryover from high to low samples is evidenced by less negative readings. The data in Table 4 reveals no systematic problem with carryover when using EMIT II or OnLine reagents on the Hitachi analyzer at these concentrations. Any sequential influence noted here appears to be more a function of the variability of both immunoassays rather than due to inefficiency of the Hitachi pipetting system. Carryover was noted for both EMIT II and OnLine when testing real samples containing extremely high drug concentrations, especially with cocaine positive samples. However, these positives carryover to fewer negative samples than with the RIA procedure. Carryover with these same samples was somewhat less with the OnLine reagents than with the EMIT II assays.



(a)

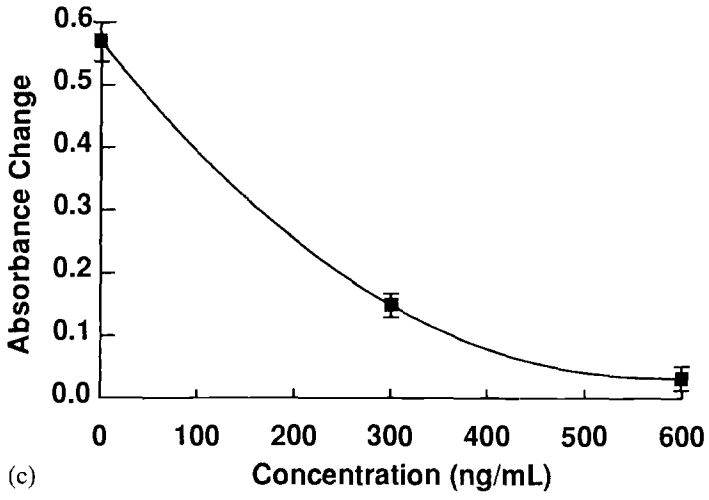


(b)

FIG. 3—*OnLine calibration curves based on averaged daily data (n = 20). Error bars represent ±2 SD. (a) THC; (b) cocaine; (c) opiate; (d) barbiturate; (e) PCP.*

Method Comparison

The Method Comparison data is given in Table 5. A total of 373 samples were confirmed positive for THC. The Abuscreen assay detected 370 and the OnLine Assay 371 of these urines and thus gave essentially equivalent results. Abuscreen detected two positive samples missed by OnLine whereas OnLine found three positives missed by Abuscreen. These five samples were all low positives, 15 to 21 ng/mL, and both assays showed reactivity. EMIT II detected 337 positive samples (90.3%). EMIT II's perform-



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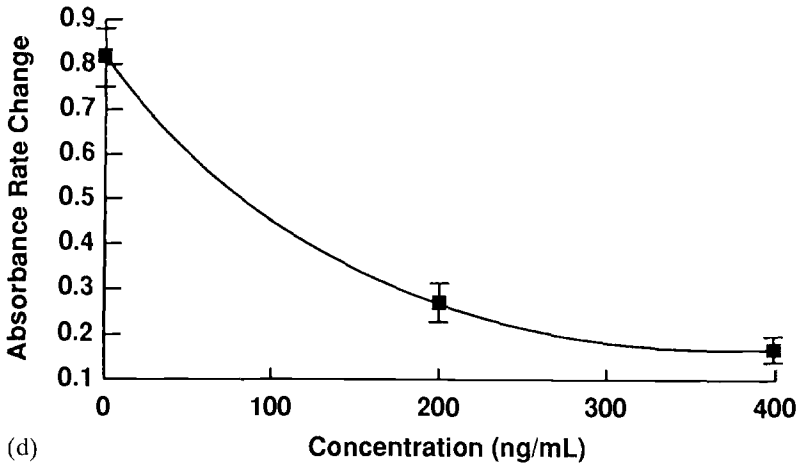
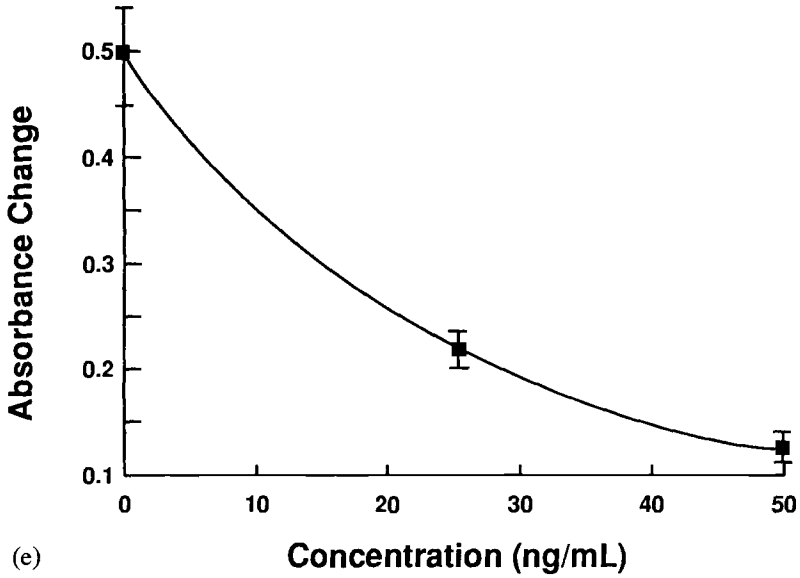


(d)

FIG. 3—*Continued.*

ance for THC in comparison to RIA was very similar to the results obtained in a previous study in which EMIT II found 88.7% of positives and EMIT 700 detected 90.6% of positives (total positives = 192) [2]. The majority of positives missed by EMIT were low positives (15-30 ng/mL) and did show reactivity below the cutoff. One sample quantitated at 79 ng/mL and gave an EMIT II result of −2, in other words, just below the cutoff. If analyzed again, at least some of these samples might very likely have yielded positive results by EMIT II.

Based on the large number of marijuana positive samples examined in this and our previous method comparison study, we conclude that the Abuscreen RIA and the OnLine assays are likely to detect about 10% more marijuana positives than the EMIT assay, either the 700 or II formulations. However, this discussion would not be complete without noting the unique marijuana calibrators used by OnLine. These calibrators consist of a racemic mixture of the d and l stereoisomers of 11-NOR-DELTA-9-THC-CARBOXYLIC acid. The Abuscreen and EMIT calibrators both consist of only the naturally occurring l isomer. It is our understanding that the OnLine antibody reacts with



(e)

FIG. 3—*Continued.*

only the l isomer while an equal amount of the d isomer is added to the calibrator to bring it to the total nominal concentration. Thus, the 50 ng/mL cutoff calibrator contains 25 ng/mL each of the isomers for a total of 50 ng/mL of the metabolite. GC/MS values for the OnLine 50 ng/mL cutoff calibrator were 44.3, 44.4, and 46.6 ng/mL. A typical Abuscreen GC/MS value for the cutoff calibrator is 51.2 ng/mL and for EMIT II, 53 ng/mL. Our laboratory's criteria for an acceptable immunoassay cutoff calibrator requires it to quantitate by GC/MS within ±10% of the nominal value, in this case, from 45–55 ng/mL of THC metabolite. The Abuscreen and EMIT II marijuana cutoff calibrators were both in this range, tending to be slightly greater than 50 ng/mL. Two out of three GC/MS values for the OnLine cutoff calibrator were below this range. The ability of an immunoassay to detect positives is enhanced if the actual concentration of the drug/drug metabolite in the cutoff calibrator is less than the nominal value. Since this study, the manufacturer has modified the OnLine marijuana calibrators and they now use only l isomer. We did not have the opportunity to use the new THC calibrator formulation but

TABLE 2—*Slopes between negative and cutoff calibrators from EMIT II, OnLine, and RIA calibration curves. EMIT II and OnLine slope values are multiplied by 10^4 and OnLine and RIA slopes are listed as positive numbers.*

	Slope		
Drug	RIA	EMIT II	OnLine
Marijuana	514	11.0	81.2
Cocaine	223	1.6	20.6
Opiates	177	1.9	13.9
Barbiturates	141	3.2	26.8
Phencyclidine	2178	27.2	111.6

TABLE 3—*Day-to-day precision of the EMIT II and OnLine negative, cutoff, and high calibrators (N = 20). Actual absorbance rate change (EMIT II) and absorbance change (OnLine) data was used.*

Drug	Calibrator	Mean Value	SD	CV (%)
		EMIT II		
Marijuana	0	.292	.007	2.4
	50	.347	.018	5.6
	100	.420	.008	1.9
Cocaine	0	.203	.003	1.5
	150	.228	.003	1.2
	3000	.354	.004	1.0
Opiates	0	.127	.003	2.0
	300	.183	.003	1.4
	1000	.251	.003	1.2
Barbiturates	0	.228	.007	3.2
	200	.291	.008	2.8
	1000	.448	.011	2.5
Phencyclidine	0	.190	.002	1.1
	25	.258	.007	2.6
	100	.317	.003	0.8
		OnLine		
Marijuana	0	1.129	.057	5.0
	50	.723	.051	7.0
	100	.49	.032	6.6
Cocaine	0	.614	.032	5.3
	150	.305	.018	5.8
	300	.145	.012	8.0
Opiates	0	.565	.022	3.9
	300	.148	.009	6.3
	600	.02	.001	6.6
Barbiturates	0	.819	.03	3.7
	200	.283	.014	4.9
	400	.171	.009	5.0
Phencyclidine	0	.500	.030	5.9
	25	.221	.006	2.8
	500	.114	.005	4.7

TABLE 4—*Carryover data for the EMIT II and OnLine assays as performed on the Hitachi 717.*

	Carryover (%)	
Drug	EMIT II	OnLine
Marijuana	+1.1	−1.9
Cocaine	−6.6	+7.6
Opiates	+0.8	+1.7
Barbiturates	+7.7	−4.1
Phencyclidine	−0.3	−0.1

TABLE 5—*Method comparison data for RIA, EMIT II, OnLine, and GC/MS assay.*

Drug	RIA POS (%)	EMIT II POS (%)	OnLine POS (%)	GC/MS POS
Marijuana	370 (99.2)	337 (90.3)	371 (99.4)	373
Cocaine	271 (99.6)	267 (98.2)	269 (98.9)	272
Opiates	136 (100)	136 (100)	136 (100)	136
Barbiturate	41 (78.8)	44 (84.6)	50 (96)	52

the manufacturer indicates that performance is equivalent to that of the original racemic mixture calibrator.

The EMIT II and OnLine cocaine assays both are comparable to the RIA procedure. All assays detected >98% of positive urines. RIA had the best detection rate (99.6%) but did miss one positive which was detected by both EMIT II and OnLine. In the cases in which EMIT II and OnLine failed to yield positive results for confirmed samples detected by RIA, both nonradioisotopic tests did show some degree of reactivity. All three immunoassays detected samples which failed to confirm (<100 ng/mL) but which did contain benzoylecgonine. There were instances in which both EMIT II and OnLine were positive but RIA was negative and the samples contained benzoylecgonine below the cutoff value. We conclude that all three cocaine immunoassays deliver essentially equivalent performance.

In the case of opiates, all three immunoassays detected all samples which confirmed positive. For the samples which failed to confirm (codeine and/or morphine <300 ng/mL), all immunoassays were positive or showed some degree of reactivity.

The greatest disparity in immunoassay performance was found for barbiturates. OnLine detected all but two confirmed positives, EMIT II missed eight, and RIA eleven samples. All positive urines contained either phenobarbital or butalbital.

As the focus of most drug testing laboratories is on detecting positive samples, it is natural to rate immunoassays in terms of how many urines confirmed by GC/MS they can detect. Typically, immunoassays will also detect some samples which do not confirm by GC/MS. Table 6 lists the screen positive but GC/MS negative samples encountered in this study by each assay. Considering that thousands of urines were screened, only a modest number of unconfirmed positives were detected and the differences among the assays are not really marked. There is a tendency to refer to these samples as "false positives" but this terminology is not appropriate in most cases. In many instances, two or all three immunoassays yielded positive results with these samples. In cases where only one screen test called a sample positive, the other two normally registered analytical signals that fell between that of the negative and cutoff calibrators. In other words, enough drug was present to cause some degree of reactivity but the response was below the below the positive threshold. For example, one sample was strongly positive by all three assays for THC but gave a GC/MS value of 7.7 ng/mL. Another sample was negative (but reactive) by RIA and EMIT II for THC, just above the cutoff by OnLine, and quantitated at 14.8 ng/mL. Thus, drug is present in these samples, just not enough to be confirmed by the more specific GC/MS procedure. In recognition of this fact, some laboratories favor reporting results as "none detected" rather than "negative" for samples screening below the cutoff value.

Method comparison data is not reported for the PCP assays as our laboratory finds so few PCP positive samples. Positive PCP internal open and blind controls are routinely tested as well as a variable number of external blind PCP positive controls. All immunoassays successfully detected these control samples except for one external blind which tested negative by EMIT II and OnLine (although both assays showed reactivity with this sample). During the evaluation period, no non-control PCP positives were detected.

TABLE 6—*Number of samples that tested positive by immunoassays but that did not confirm positive by GC/MS using the appropriate DOD cutoffs.*

Drug	RIA	EMIT II	OnLine
Marijuana	27	15	18
Cocaine	11	13	14
Opiates	22	22	19
Barbiturates	0	5	6

Also conspicuously missing from this report is any discussion of amphetamine immunoassays. Both EMIT II and OnLine assays were evaluated in comparison to the Abuscreen methamphetamine test. Amphetamine screening has traditionally been a problematical activity and our data underscores the variability that can be expected for these immunoassays, in short, agreement among the three assays was not as good as for the other tests with a number of unconfirmed amphetamine positives by all three tests. This data will be reported separately at a later time.

We are not aware of many reports in the literature which have compared immunoassays for such a large number of samples. Our data is consistent with that of other investigators. Frederick and Green compared two EMIT and two RIA marijuana tests and found discrepant results, attributable mainly to differences in the calibrators and cutoffs used by the assays [*5*]. Abercromble and Jewell compared an EMIT marijuana field screening system with the Abuscreen Marijuana Test [*6*]. In contrast to our findings, the EMIT screening assay detected more presumptive positives than the RIA test. However, when the two tests are compared on the basis of the number of GC/MS confirmed positives (n = 160, cutoff = 20 ng/mL), Abuscreen detected all 160 positives and EMIT 144 (90%). The confirmed positive rate is amazingly close to ours for EMIT II (90.3%). Wells and Barnhill compared three RIA marijuana assays (including Abuscreen) with EMIT [*7*]. They found discrepancies among the RIA results but noted that RIA consistently detected a greater proportion of positives than EMIT. Even though all of the immunoassays used a 100 ng/mL cutoff, equivalent screening results were not obtained for marijuana. Cone and Mitchell compared three RIA cocaine tests with two EMIT cocaine assays [*8*]. All samples which confirmed by GC/MS (n = 61) screened positive by the EMIT and the RIA tests (including the Abuscreen assay). Their findings are consistent with our observation of equivalent performance by EMIT and RIA for cocaine screening.

When comparing any immunoassay system to another, the type of instrumentation or analyzer coupled with the reagents can significantly affect the results. For RIA, the type and quality of the manual pipettes and/or automated pipetting system and the gamma counter are significant factors. For EMIT and OnLine, the specific automated analyzer is critical. A definite strength of both EMIT and OnLine is that they can be adapted to a wide variety of analyzers. The Hitachi 717 is certainly commonly used with EMIT reagents and produces quite satisfactory results. While OnLine reagents are not yet widely used, they work very well on the Hitachi 717. Performance of either immunoassay could be somewhat better or worse than reported here with a different analyzer and similar performance can be expected with a different 700 series Hitachi analyzer.

The data presented here and by other reports in the literature show that all immunoassays do not perform equally well for some drugs. When selecting a screening procedure, a laboratory must make practical decisions aimed at balancing the operational characteristics of immunoassays against their ability to detect confirmable positive samples.

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Address requests for reprints or additional information to
David A. Armbruster
Air Force Drug Testing Laboratory
AL/AOTQ 2601 West Road, Suite 1
Brooks AFB
Texas 78235