TECHNICAL NOTE

Mary J. Fyfe,[1] Ph.D.; P. Chand,[1] Ph.D.; C. McCutchen,[1] B.S.; J. Shelton Long,[1] B.S.; A. S. Walia,[1] Ph.D.; C. Edwards,[1,2] B.S.; and Ray H. Liu,[1,2] Ph.D.

Evaluation of Enzyme Immunoassay Performance Characteristics—Phencyclidine Example

REFERENCE: Fyfe, M. J., Chand, P., McCutchen, C., Long, J. S., Walia, A. S., Edwards, C., and Liu, R. H., **"Evaluation of Enzyme Immunoassay Performance Characteristics—Phencyclidine Example,"** *Journal of Forensic Sciences*, JFSCA, Vol. 38, No. 1, January 1993, pp. 156–164.

ABSTRACT: Four reagent formulations (three provided by a manufacturer; one prepared in-house by mixing equal volumes of two commercial reagents) are used for the assay of phencyclidine (PCP) in urine samples. Performance characteristics evaluated included assay precision and sensitivity at and near the assay cutoff concentration. Data resulting from the reagent prepared in-house are better than those using then commercially available formulations, and are comparable with those obtained using the recently available new commercial formulation.

KEYWORDS: toxicology, drug testing, enzyme immunoassay, phencyclidine

A drug of common abuse can often be tested by the same methodology using reagents provided by various manufacturers. Furthermore, a single manufacturer may supply various formulations suitable for different analyzer and test specificity requirements. Thus, it is interesting to compare the performance characteristics of various reagents.

Several studies on specific protocols have been reported [*1–3*]. We wish to report a comparative study using the Reply™ Automated Chemistry Analyzer (Olympus Corporation, Lake Success, NY) with three reagent formulations obtained from Syva Company (Palo Alto, CA) for the assay of phencyclidine (PCP) in urine samples. We also experimented a reagent formulation prepared in-house by 1:1 (v/v) mixing of two different Syva formulations.

Performance characteristics evaluated included assay precision and sensitivity at and near the assay cutoff concentration.

Received for publication 4 April 1992; revised manuscript received 15 June 1992; accepted for publication 29 June 1992.

[1]Co-Technical Director, Co-Technical Director, Technical Staff, Operation Director, Senior Vice-President, Technical Staff, and Senior Consultant, respectively, Environmental Chemical Corporation, Birmingham, AL.

[2]Graduate Student and Professor and Director, Graduate Program in Forensic Science, Department of Criminal Justice, University of Alabama at Birmingham, Birmingham, AL.

Materials and Methods

Materials

The following assay kits were obtained from Syva Company (Palo Alto, CA): Emit® d.a.u.™ Phencyclidine Assay kit, including Emit® d.a.u.™ Phencyclidine Assay Antibody/Substrate Reagent A, Enzyme Reagent B, Emit® Drug Assay Buffer Concentrate, Emit® d.a.u.™ Negative Calibrator, Emit® d.a.u.™ Low Calibrator B, and Emit® d.a.u™ Medium Calibrator B; Emit® 700 Phencyclidine Assay kit, including Emit® 700 Phencyclidine Assay Antibody/Substrate Reagent 1, Enzyme Reagent 2, Emit® 700 Calibrator B, and Emit® 700 Control Set B (Positive Control B and Negative Control B); and Emit® II Phencyclidine Assay kit, including Emit® II Phencyclidine Assay Antibody/Substrate Reagent 1, Enzyme Reagent 2, Emit® Calibrator Level 0, and Emit® Calibrator A Level 1 (cutoff), Emit® Calibrator A Level 2 (high).

Reply™ Automated Chemistry Analyzer from Olympus Corporation (Lake Success, NY) was used for all assays.

Experimental Design

Four test protocols were conducted. Three of these protocols were those recommended by the manufacturer for their assay kits [*4–6*]: Emit® d.a.u.™ Phencyclidine Assay ($EMIT_{dau}$), Emit® 700 Phencyclidine Assay ($EMIT_{700}$), Emit® II Phencyclidine Assay ($EMIT_{II}$). The fourth protocol ($EMIT_{mixed}$) used 1:1 (v/v) mixing of Antibody/Substrate Reagent A with Antibody/Substrate Reagent 1 and Enzyme Reagent B with Enzyme Reagent 2 from Emit® d.a.u.™ Phencyclidine and Emit® 700 Phencyclidine Assay kits, respectively.

Sample, reagent, and diluent volumes recommended by the Reply™ manufacturer were used for all protocols, specifically, the sample/diluent and the reagent/diluent (1-step; 2-step) volumes for the $EMIT_{dau}$, $EMIT_{700}$, and $EMIT_{mixed}$ protocols are: sample/diluent, 20/20; and reagent/diluent: 130/30, 130/30. The parallel parameters used for the $EMIT_{II}$ protocol are 8/5, 135/15, and 135/15, respectively.

Results and Discussion

Perhaps the most important characteristic of a preliminary screen test is its ability to correctly differentiate samples containing the analyte at or above the cutoff concentration (25 ng/mL for PCP) from those containing less or no analyte. To meet this requirement, the assay should provide acceptable *sensitivity* and *precision* in the concentration range of concern. (Sensitivity is defined as the assay response per unit analyte concentration change, or simply, the slope of the dose-response plot.)

Assay Sensitivity

Visual inspection of Fig. 1a ($EMIT_{700}$) and 1b ($EMIT_{dau}$) reveals the following performance characteristics:

1. The signal-dose plot of the $EMIT_{700}$ protocol in the 0 to 25 ng/mL range is linear with a steep slope, but becomes flat in the 25 to 50 ng/mL range; thus, the operation parameters recommended by Reply™ manufacturer for the $EMIT_{dau}$ protocol cannot be used for the $EMIT_{700}$ assay;
2. The signal-dose plot of the $EMIT_{dau}$ is linear in the 0 to 50 ng/mL range with a response change (or "separation") of only about 60 units, resulting in a smaller slope of the plot.

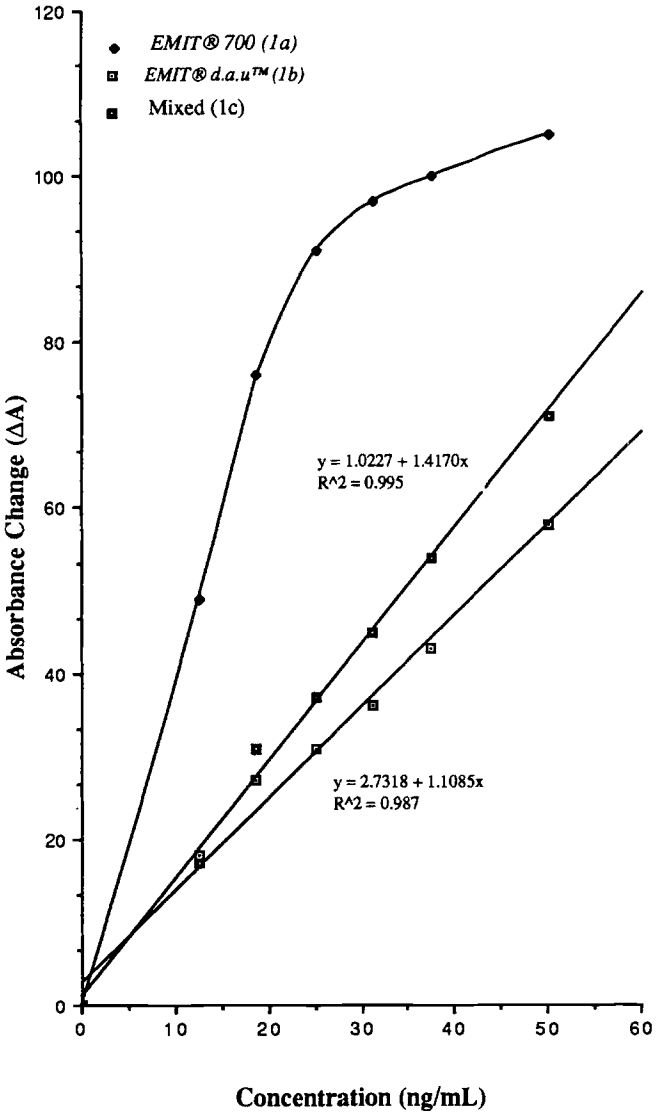

FIG. 1—*Absorbance change vs. concentration plots using the* $EMIT_{700}$ *(1-a), the* $EMIT_{dau}$ *(1-b), and the* $EMIT_{mixed}$ *(1-c) protocols.*

It was then rationalized that the analyte/reagent ratio of neither protocol is optimized for the targeted 0 to 50 ng/mL analyte concentration range, and that a more appropriate ratio may be obtained by mixing these two reagents. Indeed, the corresponding data (Fig. 1c) observed for the $EMIT_{mixed}$ protocol show improvement of assay sensitivity in increasing approximately 15 units of separation in the same concentration range.

Assay Precision

The sensitivity (of an assay protocol) that is required to effectively differentiate samples with a small concentration difference depends on the precision that can be achieved by

the assay; if the assay precision is high, the sensitivity become less critical. Parameters that can be effectively used for the evaluation of this aspect of performance characteristics are means and standard deviations (SD) obtained from samples with the analyte slightly above, at, or slightly below the cutoff concentration.

Individual data points obtained from within-run and between-day runs using the $EMIT_{mixed}$ protocols are compared in Tables 1 and 2. Individual data points obtained from within-run using the recently available product (Emit® II Phencyclidine Assay) are shown in Table 3, and compared with the corresponding data obtained from the $EMIT_{mixed}$ protocol in Fig. 2.

TABLE 1—*Within-run ΔA readings and statistical data ($EMIT_{mixed}$ protocol).*

	Concentration (ng/mL Phencyclidine)						
	Neg	12.5	18.8	25.0	31.3	37.5	50.0
1	201	223	237	243	250	258	275
2	206	224	235	243	251	260	276
3	207	221	234	242	251	258	274
4	205	225	236	245	253	261	277
5	206	221	238	243	251	260	277
6	204	224	237	241	252	261	277
7	207	221	237	245	250	260	275
8	206	222	237	243	250	259	277
9	206	225	237	242	251	260	276
10	203	221	236	242	249	259	279
11	206	224	235	243.	250	258	277
12	206	223	238	243	250	259	275
13	203	222	237	241	249	261	275
14	204	222	237	242	251	260	277
15	205	224	235	244	251	259	278
Ave	205	222	236	242	250	259	276
SD	1.69	1.47	1.18	1.20	1.05	1.06	1.34
%CV	0.82	0.66	0.50	0.49	0.42	0.40	0.48
Difference from neg.	—	17	31	37	45	54	71

TABLE 2—*ΔA readings and statistical data of 12 batches run on 10 different days ($EMIT_{mixed}$ protocol).*

	Concentration (ng/mL Phencyclidine)					
Date	Neg	12.5	25.0	31.3	37.5	50.0
5/28	180	203	213	221	235	248
5/28	177	201	214	225	235	254
5/30	181	203	216	223	235	248
5/31	179	197	213	222	229	246
6/02	177	192	206	216	223	240
6/13	189	209	226	234	242	258
6/14	187	205	221	231	238	255
6/16	183	205	218	229	237	254
6/18	186	205	219	229	239	257
6/19	184	203	219	227	237	254
6/20	184	203	216	228	241	253
6/20	188	208	224	234	244	259
Ave	183	203	217	227	236	252
SD	4.14	4.60	5.38	5.42	5.72	5.59
%CV	2.26	2.27	2.48	2.39	2.42	2.21
Difference from neg.	—	20	34	44	53	69

TABLE 3—*Within-run ΔA readings and statistical data ($EMIT_{II}$ protocol).*

	Concentration (ng/mL Phencyclidine)						
	Neg	12.5	18.8	25.0	31.3	50.0	75.0
1	163	175	186	196	205	232	247
2	162	174	186	195	208	235	251
3	161	176	185	193	207	235	250
4	161	175	183	194	208	233	248
5	161	173	184	194	208	233	251
6	161	177	185	196	204	231	249
7	160	177	183	193	206	232	248
8	162	175	185	194	204	233	247
9	161	176	182	192	206	233	246
10	159	174	185	195	207	232	245
11	161	175	183	193	203	232	250
12	161	177	185	195	206	232	248
13	161	173	184	193	205	230	246
14	158	172	179	191	202	229	244
Ave	161	175	184	194	206	232	248
SD	1.23	1.59	1.86	1.46	1.91	1.64	2.18
%CV	0.76	0.91	1.01	0.75	0.93	0.71	0.88
Difference from neg.	—	14	23	33	45	71	87

The mean and standard deviation data resulting from the $EMIT_{dau}$, $EMIT_{mixed}$, and the $EMIT_{II}$ protocols as shown in Table 4.

Separation

The ability of an assay protocol to separate samples with different concentrations are evaluated on the overlapping (or nonoverlapping) characteristics between the following standards: 18.8 ng/mL (25% below cutoff) and 25 ng/mL (cutoff); and 25 ng/mL (cutoff) and 31.2 ng/mL (25% above cutoff). Corresponding data calculated from the $EMIT_{dau}$, $EMIT_{mixed}$, protocols and the $EMIT_{II}$ are shown in Table 5.

Data in Table 5 indicate that means of the paired standards are separated by more than 2 standard deviations with the $EMIT_{mixed}$ and $EMIT_{II}$ protocols, but not the $EMIT_{dau}$ protocol. Thus, the probability in differentiating (by the $EMIT_{mixed}$ and $EMIT_{dau}$ protocols) samples with concentrations 25% below or above the cutoff concentration from the cutoff is better than 97.72%. The $EMIT_{mixed}$ protocol was applied to test samples and found to correctly identify all positive samples as shown in Table 6.

Deviation from Manufacturer's Recommended Procedure

The improved performance resulting from mixing two reagent formulations provided by the same manufacturer is itself an interesting observation worth reporting. From a practicing point of view, the established protocol was essential for effective use of the analyzer for PCP enzyme immunoassay at the time the study was conducted—$EMIT_{II}$ was then not yet available. Now that $EMIT_{II}$ formulation is available, this reported protocol provides an alternative approach with lower reagent cost.

For many laboratories, the most frequently encountered standards and criteria are those set forth by the National Laboratory Certification Program (NLCP) administered by the National Institute on Drug Abuse. On this matter, the NLCP has advised that

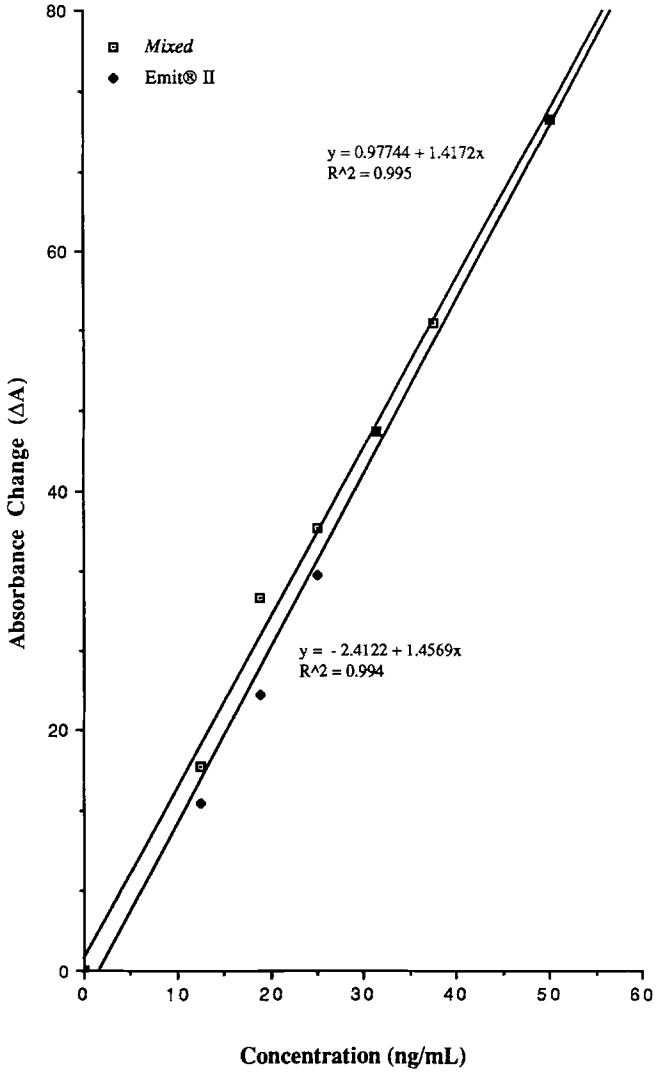

FIG. 2—*Comparison of the* $EMIT_{mixed}$ *and the* $EMIT_{II}$ *protocols.*

supporting changes to the manufacturers' procedures should, at a minimum, be characterized by data which defines the assay's linearity, precision, accuracy, detection limits, and specificity [*8*]. The laboratory should also demonstrate that the assay can differentiate between positive and negative specimens. Data reported herein were generated in the course of meeting these criteria.

Conclusion

With the operation parameters recommended by the Reply™ manufacturer for the $EMIT_{dau}$ protocol, the $EMIT_{mixed}$ protocol provides better performance characteristics (linearity and sensitivity) than the $EMIT_{dau}$ and $EMIT_{700}$ protocols in the 0 to 50 ng/mL range. Samples with phencyclidine concentrations at 25% below or above the cutoff

TABLE 4—*Mean and precision data of the $EMIT_{dau}$, $EMIT_{mixed}$, and $EMIT_{II}$ protocols.*

	Concentration, mean ΔA (Ave), and standard deviation (SD)													
	0 ng/mL		12.5 ng/mL		18.8 ng/mL		25 ng/mL		31.3 ng/mL		37.5 ng/mL		50 ng/mL	
Protocol	Ave	SD	Ave	SD	Ave	SD	Ave	SD	Ave	SD	Ave	SD	Ave	SD
$EMIT^{a}_{mixed}$	205	1.68	222	1.47	236	1.18	242	1.20	250	1.05	259	1.06	276	1.34
$EMIT^{b}_{dau}$	194	3.05	213	2.66	221	1.96	224	2.20	230	1.31	237	1.51	252	1.52
$EMIT^{c}_{II}$	161	1.23	175	1.59	184	1.86	194	1.46	206	1.91	—	—	232	1.64

[a]Means and standard deviations of 15 measurements.
[b]Means and standard deviations of 3 measurements for 0 and 50 ng/mL, and of 8 measurements for other concentrations.
[c]Means and standard deviations of 14 measurements.

TABLE 5—*Comparison of critical separations obtained from the $EMIT_{dau}$, the $EMIT_{mixed}$, and the $EMIT_{II}$ protocols.*

Protocol	Concentration and mean ΔA plus and/or minus 2 standard deviation (SD) 18.8 ng/mL [Mean + 2 SD]	25 ng/mL [Mean − 2 SD]	25 ng/mL [Mean + 2 SD]	31.3 ng/mL [Mean − 2 SD]
$EMIT_{mixed}$	238	240	244	248
$EMIT_{dau}$	225	220	228	227
$EMIT_{II}$	178	180	188	191

TABLE 6—*Application of the $EMIT_{mixed}$ protocol.*

Sample	GC/MS Conc. (ng/mL)	ΔA	EIA Result[a,b]
1	0	202	Negative
2	0	195	Negative
3	31	246	Positive
4	0	200	Negative
5	0	198	Negative
6	32	245	Positive
7	0	205	Negative
8	0	204	Negative
9	34	245	Positive
10	0	194	Negative
11	0	205	Negative
12	32	247	Positive
13	31.3 (Control)	249	Positive
14	37.5 (Control)	258	Positive
15	31.3 (Control)	251	Positive
16	37.5 (Control)	259	Positive
17	0	204	Negative
18	0	192	Negative
19	39	256	Positive
20	37	253	Positive

[a]Individual readings, mean, standard deviation, and %CV for the cutoff standards of the batch are: 237, 235, 231; 234; 3.06; and 1.31%, respectively. Thus, 234 was used as the cutoff value.

[b]Individual readings, mean, standard deviation, and %CV for the negative standards of the batch are: 204, 195, 199; 199; 4.51; and 2.27%, respectively.

standard (25 ng/mL) can be differentiated from the cutoff with a probability better than 97.72% (2 standard deviation). Results obtained using the $EMIT_{mixed}$ protocol are comparable with those obtained using the recently available $EMIT_{II}$ protocol.

References

[1] ElSohly, M. A. and Sanford D. F., "Cutoff of 25 ng/mL for EMIT d.a.u. Phencyclidine Assay," *Journal of Analytical Toxicology*, Vol. 14, 1990, pp. 192–193.

[2] Cary P. L., Johnson C. A., Folsom, T. M., and Bales, W. R., "Immunoassay Method Validation for a Modified EMIT Phencyclidine Assay," *Journal of Analytical Toxicology*, Vol. 16, 1992, pp. 48–51.

[3] Saffarian, A., Fairchild, L., and Haley, J., "Development of an Emit® d.a.u.™ 25 ng/ml Phencyclidine Assay on the Syva® ETS® System," presented at the 44th Annual Meeting of the American Academy of Forensic Science, New Orleans, LA, Feb. 1992.

[4] "Emit® d.a.u™ Phencyclidine Assay" instructions, Syva Company, Palo Alto, CA, May 1988.

[5] "Emit® 700 Phencyclidine Assay" instructions, Syva Company, Palo Alto, CA, June 1990.

[6] "Emit® II Phencyclidine Assay" instructions, Syva Company, Palo Alto, CA, Mar. 1991.
[7] Reply Methodology Summary, Operation Manual, Reply™ Automated Chemistry Analyzer, Olympus Corporation, Lake Success, NY, Sept. 1, 1989.
[8] Williams D. R., "An Overview of Test Method Validation," *BioPharm Manufacturing*, Vol. 1, No. 00, 1987, pp. 34–51.

Address requests for reprints or additional information to
Ray H. Liu, Ph.D.
Environmental Chemical Corporation, Inc.
1075 South 13th St.
Birmingham, AL 35205