Common Commercial Immunoassay for Workplace Drug Urinalysis — Principle, Cross-Reactivity and Interference

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ABSTRACT

The underlying principles of the commonly used radioimmunoassay, enzyme immunoassay, fluorescence polarization immunoassay, and "particle" immunoassay are outlined. Cross-reacting characteristics of these immunoassays as reported by the manufacturers and independent laboratories are tabulated. These information show that commercial immunoassay kits for drug categories that are included in workplace drug urinalysis programs are generally more specific than those kits that are for clinical uses only. Furthermore, recently manufactured immunoassay kits targeted for use in workplace drug urinalysis programs are more specific than those manufactured earlier.

Reported effects of adulterants, such as salt, cleaning agents. etc., on commonly used immunoassays are summarized. Without more comprehensive and systematic studies, it is difficult to make general statements concerning the superiority of one methodology over the others. It is clear, however, that cannabinoid assays are most susceptible to the influence of adulterants.

Key words : Immunoassay, Cross-reactivity, Interference, Urine.

INTRODUCTION

Based on the definiteness of its analytical finding, an analytical procedure can be considered a preliminary or a conclusive method. Preliminary methods of analysis are useful for screening purposes. They can be used in the field where instrumentation needed for more definite methods of analysis cannot be conveniently applied. Typical examples of this category of methods include color tests and portable immunoassay kits, such as Abuscreen® ONTRAK™ (Roche Diagnostic Systems: Branchburg, NJ), *accu* PINCH (HYCOR Biomedical: Garden Grove, CA), MACH IV® (Drug Screening Systems: Blackwood, NJ), I.D. BLOCK™(International Diagnostic Systems: St. Joseph, MI), and Triage™panel (Biosite Diagnostics, San Diego, CA).

Preliminary methods of analysis that require major instrumentation and can only be performed under a laboratory environment may still find wide usage if they can be applied cost effectively to process a large number of samples. Typical examples of this category of methods are the immunoassays now routinely used in workplace drug urinalysis laboratories, including radioimmunoassays (RIA) marketed by Roche Diagnostic Systems (Branchburg, NJ), Diagnostic Products (Los Angeles, CA), and Immunalysis Corporation (Glendale, CA); enzyme

immunoassay (EIA) marketed by Syva Company (Palo Alto, CA); fluorescence polarization immunoassay (FPIA) marketed by Abbott Laboratories (Irving, TX); and "Particle" immunoassay (PIA) marketed by Roche Diagnostic Systems (Branchburg, NJ).

The value of a screen test procedure is not conventionally judged by its specificity toward a specific drug. Preliminary screen procedures with broad cross-reactivity are very valuable when used for emergency room drug screen or used for detecting drugs with similar functional group in cases (a) where the exact structure of the drug is not known, as often encountered in designer drug related cases; and (b) where the exact metabolites of a drug in a biological fluid has not yet been established. Positive results derived from the assay's cross-reactivity toward these unknowns allow the detection of relevant drugs without conducting multiple screen tests.

Specificity, however, is of the primary concern when a screen test procedure is used in a workplace drug urinalysis program. Current practices of workplace drug urinalysis require a two-step test protocol in which an immunoassay is used to identify "presumptive positive" samples that generate responses equivalent to or above that generated by a targeted analyte at a "cutoff" concentrations. These "presumptive positive" samples are furthered tested for specific drugs/metabolites by GC/MS procedures. Only those samples that are confirmed to include those targeted drugs (at or above "cutoff" concentrations) can be reported as positive. The analytes targeted and the cutoff concentrations of immunoassays and GC/MS adopted by major workplace drug urinalysis programs are listed in Table 1[1,2].

Table 1. Cutoff levels[a] of immunoassays and GC/MS tests adopted by the U.S. laboratory certification programs

| Drug category | Immunoassay cutoff (ng/mL) | | | GC/MS Test cutoff (ng/mL) | | |
	Analyte targeted	D_oD[b]	HHS[b]	Analyte targeted	D_oD	HHS
Amphetamine	Amphetamine	500	500	Amphetamine	500	500
Methamphetamine	Methamphetamine	500	500	Methamphetamine[c]	500	500
Barbiturates	Secobarbital	200	—[d]	Butalbital	200	—[d]
				Amobarbital	200	—[d]
				Pentobarbital	200	—[d]
				Secobarbital	200	—[d]
Cocaine	Benzoylecgonine	150	300	Benzoylecgonine	100	150
Opiates	Morphine	300	300	Codeine	300	300
				Morphine	300	300
Phencyclidine	Phencyclidine	25	25	Phencyclidine	25	25
Cannabinoids	9-THC-COOH[e]	50	100	9-THC-COOH	15	15
LSD[f]	LSD	0.5	—[d]	LSD	0.2	—[d]

[a]As of Aug. 1993.

[b]DoD: U.S. Department of Defense; HHS: U.S. Department of Health and Human Services.

[c]The sample should also contain 200 ng/mL of amphetamine to report positive for methamphetamine[3]. Additional testing to differentiate d-and l-methamphetamine may be required.

[d]Testing not included by the HHS program.

[e]9-THC-COOH: 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid.

[f]LSD: lysergic acid diethylamide.

With this two-step test protocol and reporting policy, a preliminary test procedure that responding to those drugs/metabolites not targeted for by the GC/MS procedures may generate "presumptive positive" results that are not confirmed by GC/MS. This article attempts to evaluate literature data concerning the cross-reacting characteristics of commercially available immunoassays kits that are commonly used in workplace drug urinalysis programs. Since systematic and full-scale evaluation of portable immunoassay kits, such as Abuscreen® ONTRA-K™, *accu* PINCH, MACH IV®, I.D. BLOCK™, and Triage™ panel, are rare, independent literature data on these products are very limited. They are not included in this review. The assay kits covered are further limited to those aiming for the detection of drugs/metabolites that are included in major workplace drug urinalysis programs.

Since only those samples that are tested positive by both the preliminary and the confirmatory tests can be reported as positive, the cutoff concentration adopted by the immunoassay should correspond well with the cutoff concentration of the compound targeted by GC/MS. Since all presumptive positive results obtained from an immunoassay require further confirmatory testing, adopting an inappropriately low immunoassay cutoff value will result in excess number of negative GC/MS results causing the overall analytical procedure financially inefficient. On the other hand, if the screening cutoff value is set too high, some positive samples may be rejected as negatives in the preliminary screening process, without being tested by GC/MS. With this in mind, attempts are made to compare immunoassays based on their abilities to generate apparent analyte concentrations that can be statistically correlated with the GC/MS analyte concentrations.

Limit of detection is normally an important parameter when analytical procedures are evaluated. It will not however, be emphasized in this review. To minimize issues that may derive from technology limitations and data interpreting variations, immunoassay cutoff concentrations set by workplace drug urinalysis programs are well above the limits of detection of commonly used immunoassays. Therefore, whether a particular immunoassay is suitable for a workplace drug urinalysis program is normally not judged by the limit of detection the immunoassay can achieve.

METHODOLOGY AND CROSS-REACTIVITY

The most important aspects of immunoassay technologies are:

1. the production of an antibody possessing the desired specificity, affinity, and sensitivity;

2. the development of a mechanism for the reaction of the specific antibody with the analyte ; and

3. the design of a detection system suitable for measuring the occurrence and extent of the specific reaction.

The antibody is produced in an animal (polyclonal) or in an organic culture (monoclonal) environment in response to an antigenic complex composed of the drug of interest coupled to a carrier protein.

Most immunoassay procedures utilize the competitive binding principle in which the antibody is allowed to react with a mixture of labeled (control) and unlabeled (sample) drugs. The presence and the quantity of a drug in the sample is evaluated based on the quantities of the labeled drug in the reacted or unreacted forms. The control drugs are labeled in different ways, each requiring different methods of detection and quantification. Thus, radioactive isotopes, such as ^{125}I, are used for labeling in radioimmunoassay (RIA) methods; active enzymes, which are capable of converting (indirectly) nicotinamide adenine dinucleotide (NAD) to nicotinamide 6-(2-aminoethylamino) purine dinucleotide (AENAD), are coupled to the drug in enzyme multiplied immunoassay techniques (EMIT); fluoresceins are coupled to the drug used in fluorescence polarization immunoassay (FPIA);

and particles are attached to the drug in particle immunoassay (PIA). [Basic operation principles and applications of these methods will be discussed later in separate sections.]

Several immunological assay systems have been developed for the detection of drugs in biological specimens. Commercial kits are available for routine use in clinical, toxicological, and forensic laboratories. Major immunoassays that have found significant applications for preliminary testing of commonly abused durgs are listed in Table 2.

Methodologies listed in Talbe 2 are considered *heterogeneous* if a phase separation step is needed prior to detection. The detection process is designed to measure the extent of the labeled antigen linked (directly or indirectly) to the antibody, thus reflecting the amount of the test drug (unlabeled antigen) in the sample. Methodologies based on the measurement of radioactivity are operated heterogeneously since the detecting device cannot differentiate the sou-

Table 2. Major classification of immunoassays and commercial products for workplace drug urinalysis

Immunoassay class	Commercial source and trade name[a]	Drug groups with commercially available assay kit[b]
Radioimmunoassay (RIA)	Roche: Abuscreen®	Amphetamine, barbiturates, benzodiazepines, cannabinoids, cocaine metabolite, lysergic acid diethylamide, methamphetamine, methaqualone, morphine, phencyclidine
	DPC: Double antibody	Amphetamine, benzodiazepines, cannabinoids
	Coat-A-Count®	Barbiturates, cocaine metabolite, fentanyl, lysergic acid diethylamide, methadone, methamphetamine, morphine, phencyclidine
	Immunalysis[c]	Cannabinoids, phencyclidine
Enzyme immunoassay[d] (EIA)	Syva: EMIT®	Amphetamine, barbiturate, benzodiazepine, cannabinoid, cocaine metabolite, methadone, methaqualone, phencyclidine, propoxyphene
Fluorescence polarization immunoassay (FPIA)	Abbott: TDx®/ADx®	Amphetamine/methamphetamine, barbiturates, benzodiazepines, cannabinoids, cocaine metabolite, methadone, opiates, phencyclidine, propoxyphene
"Particle" immunoassay[e] (PIA)	Roche: Abuscreen® Online™	Amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine metabolite, methadone, opiates, phencyclidine

[a]Commercial manufacturers: Roche: Roche Diagnostic Systems (Branchburg, NJ); DPC: Diagnostic Products Corporation (Los Angeles, CA); Immunalysis: Immunalysis Corporation (Glendale, CA); Syva: Syva Company (Palo Alto, CA); Abbott Laboratories (Irving, TX).

[b]As of July 1993.

[c]Several other assay kits that were made available by this manufacturer in the past (see Table 3) are currently being reintroduced and evaluated.

[d]Enzyme immunoassay reagents from numerous sources have recently become available. These recently available reagents are not included mainly because the lack of literature data resulting from evaluations performed by independent laboratories.

[e]To the author's knowledge, the term "particle immunoassay" has not been used in the literature. In parallel with the nomenclature convention applied to FIA, EIA, and FPIA, the term "particle immunoassay" has not been used in the literature. In parallel with the nomenclature convention applied to FIA, EIA, and FPIA, the term "particle immunoassay" (PIA) is hereby adopted for immunoassays that utilize particles of various nature and sizes as the label and the basis of detection mechanism.

rce of the radioactivity (free or bound labeled antigen). Therefore, the separation step is required.

Methodologies based on the change of optical intensities do not require the separation step if these properties are modified through the substrate's linkage (directly or indirectly) to the antibody. These immunoassays can be operated without a separation step and are considered *homogeneous* immunoassays.

Both heterogeneous and homogeneous immunoassays are used for workplace drug urinalysis. Basic methodologies and cross-reactivities of the most common commercial products are further discussed below.
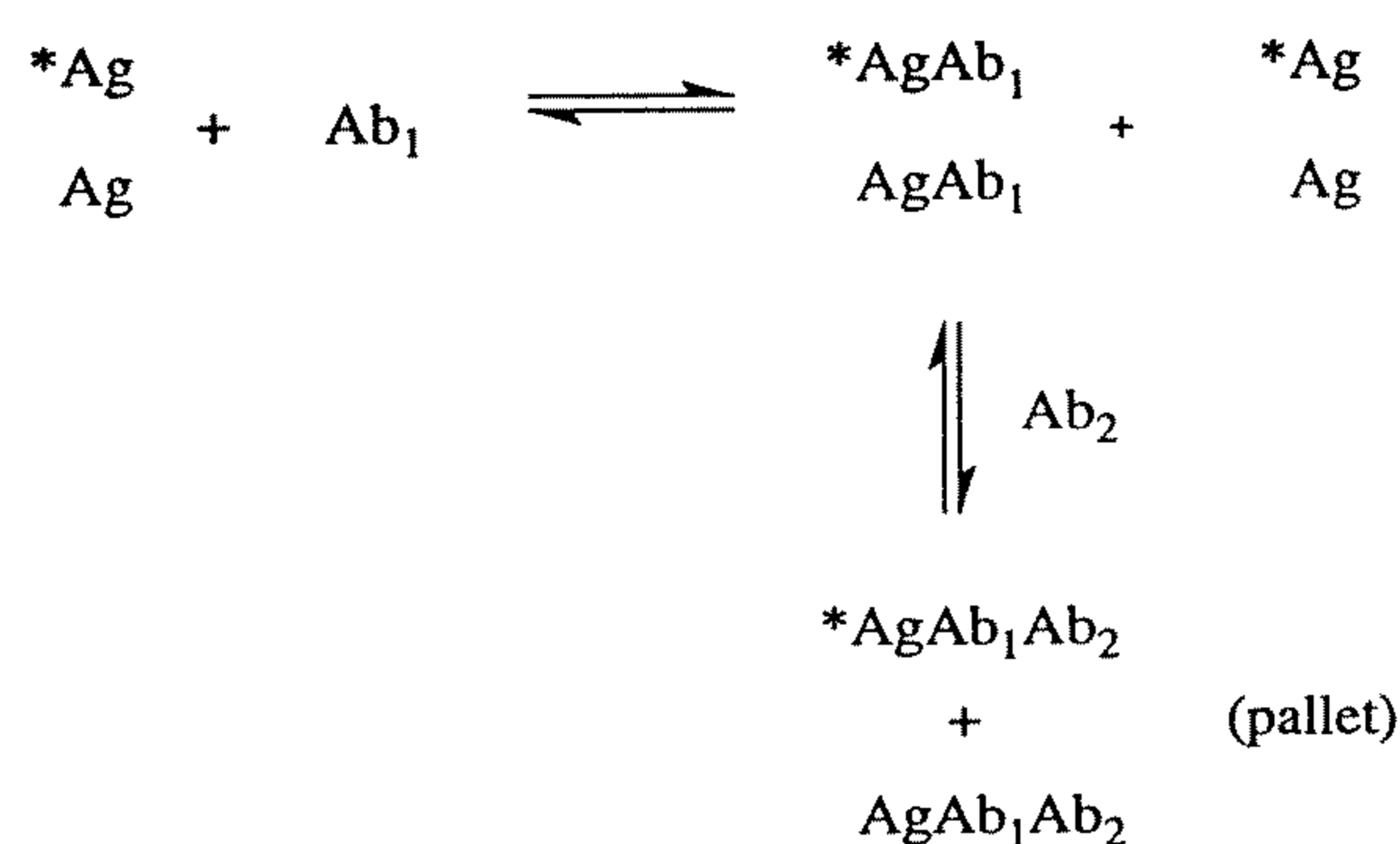
I . Radioimmunoassay

(I). Basic Methodology

Radioimmunoassay represents one of the most powerful and earliest immunoassay technologies used for high-volume drug screen. Most commonly used RIA methods are based on the competitive binding of ^{125}I-labeled antigen and free unlabeled antigen (analyte), in proportion to their concentrations, to a limited amount of antibody in the reaction mixture as shown in the first part of Figure 1.

To assess the drug's presence and its concentration in a sample, the radioactivity measured from the sample's antibody-bound fraction is compared with data established by a series of standards. Since the detection mechanism cannot differentiate the sources of the radioactivity, it is necessary to separate the antibody-bound (reaction product) from the antibody-free (unreacted) radiolabeled drug prior to radioactivity counting.

Among various processes used for phase separation, Abuscreen® and Immunalysis kits use a precipitating second antibody, Ab_2, to remove the antibody-bound fraction from the reaction mixture as shown by the second part of Figure 1. Newer reagents provided by these manufacturers allow for the addition of Ab_1 and

$$*Ag + Ab_1 \rightleftharpoons *AgAb_1 + *Ag$$
$$Ag \qquad\qquad AgAb_1 \qquad Ag$$

$$\downarrow Ab_2$$

$$*AgAb_1Ab_2$$
$$+ \qquad (pallet)$$
$$AgAb_1Ab_2$$

Figure 1. Schematic illustration of competitive immunoassay. * Ag represents the labeled form of the drug; Ag the drug (analyte) to be measured; Ab_1 the antibody, the limiting factor in the reaction, capable of binding the drug; Ab_2 the second antibody capable of binding Ab_1 to form aggregates.

Ab_2 at the same time, thus, greatly simplify the test protocol. Coat-A-Count® kits utilize immobilized antibody on the wall of a polypropylene tube, thus making the separation step automatic.

Various dose-response curves[4] established by control samples with known amounts of the drug can be used for the attempted quantification of the drug in an unknown sample. A typical standard curve is obtained by plotting B/B_0 (counts obtained from the test sample related to that from the zero dose control) against increasing concentrations of the analyte in the set of control samples (Figure 2). Another popular dose-response curve plots logit (B/B_0) against $\log_e$ [Ag], where [Ag] is the concentration of the analyte.

$$\log_e[(B/B_0)/(1-B/B_0)] = a + b \log_e [Ag] \quad (1)$$

The latter plot is advantageous in that a linear relationship of the two parameters can be obtained within a limited concentration range.

(II). Cross-reactivities

Cross-reactivity data of common commercial kits are summarized in Table 3. Most of these data are directly taken from the respective reagent package inserts ,while others are reported by independent laboratories.
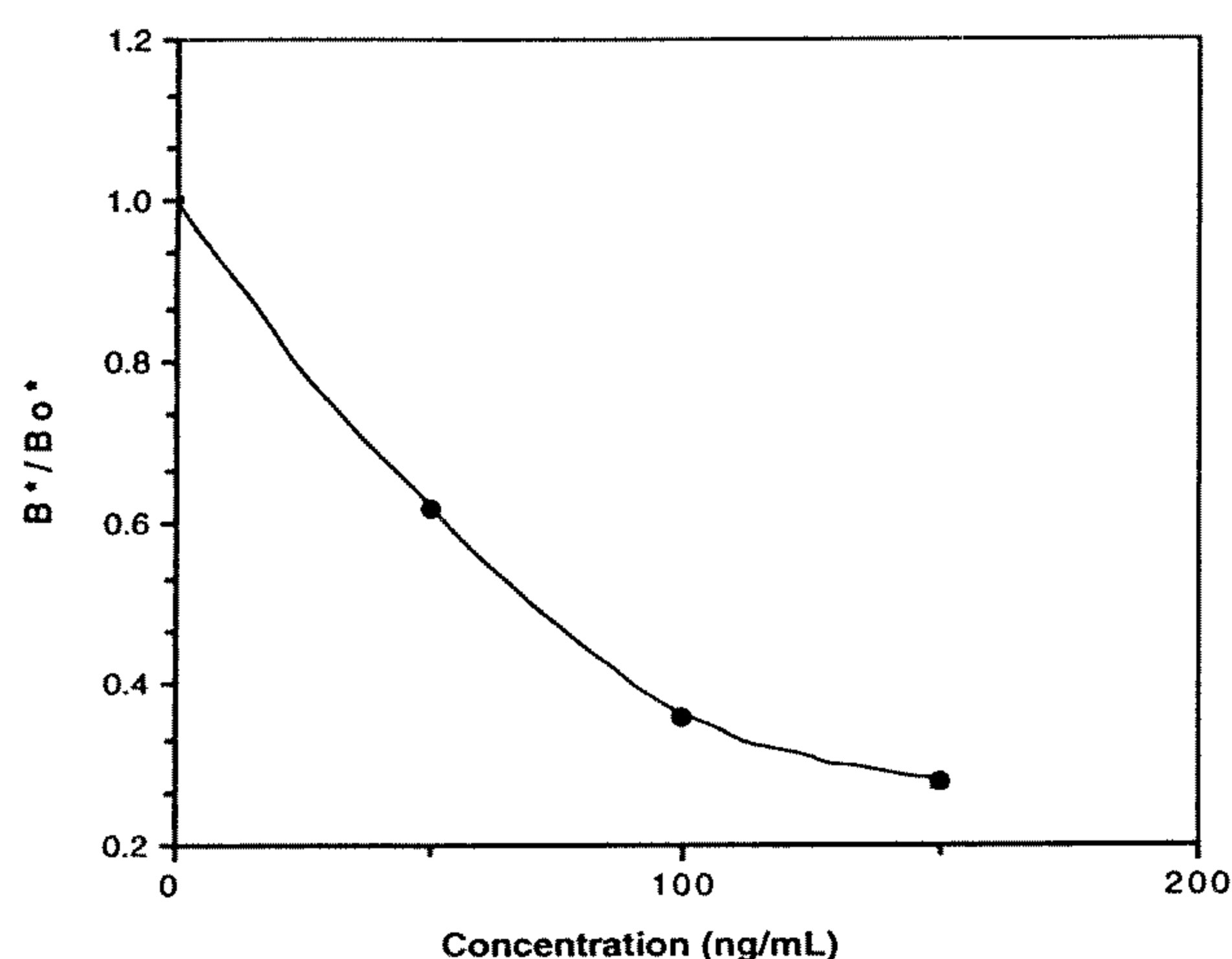
Figure 2. Dose-response curve for radioimmunoassay. (Data taken from Abuscreen® Radioimmunoassay for Phencyclidine package insert dated Oct. 1988.)

The two-step test protocol and the test result reporting policy adopted by workplace drug urinalysis programs have motivated RIA reagent manufacturers to develop and produce assay kits that will generate test results closely related to the GC/MS test results of a very limited number of targeted drugs. Despite the fact that cross-reactivity with structurally-related drugs may serve other purposes well, the trend in the RIA reagent manufacturing industry has been in increasing reagent specificities toward the very limited number of targeted drugs. This trend is clearly demonstrated by the differences in reagent specificity (as shown in Table 3) for drugs that are and are not adopted for monitoring in workplace drug urinalysis programs. Thus, highly specific kits for amphetamine, methamphetamine, benzoylecgonine, 11-nor-$\triangle^9$-tetrahydrocannabinol-9-carboxylic acid (9-THC-COOH), morphine/codeine, and phencyclidine are available. To demonstrate this point further, data shown in Table 4 indicate that earlier reagents from the same manufacturers were not as specific as current ones.

II.*Enzyme Immunoassay*

(I). *Basic Methodolgy*

Enzyme multiplied immunoassay technique (EMIT®) represents the most widely used enzyme immunoassay (EIA) technology applied to drug screen. Emit® is based on the absorbance change (at 340 nm) caused by the reduction of NAD to NADH. This reaction is coupled by the oxidation of glucose-6-phosphate to 6-phosphogluconolactone as shown in Figure 3. The latter oxidation is catalyzed by glucose-6-phosphate dehydrogense (G6P-DH) attached to the free, but not the bound, antigen.

In practice, a standard amount of the enzyme-labeled antigen and a constant limiting quantity of the antibody are used in every standard and test sample. Under the competitive reaction process, the concentration level of the analyte in a test sample will determine the amount of the enzyme-labeled antigen that remains unbound. The amount of the enzyme-labeled antigen that remains unbound will determine the oxidation rate of glucose-6-phosphate to 6-phosphogluconolactone and thus indirectly determines the absorbance change caused by the reduction of NAD to NADH. Since physical separation of the bound from the unbound antigen is not necessary for the measurement of absorption change, this is a homogeneous immunoassay. A typical dose-response curve obtained from calibrators is shown as Figure 4.

Enzyme immunoassay has also been applied for screening fentanyl[15], cocaine[16], and other drugs[17] and metabolites using a heterogeneous approach called enzyme-linked immunosorbent assay (ELISA). In the first two applications , a fixed amount of the analyte coated on the solid-phase medium competes with the analyte in the test sample to react with the first antibody added. The solid-phase medium is then washed, and an enzyme-labeled second antibody is added . The solid-phase medium is washed again, followed by the addition of an enzyme substrate. The extent of the substrate reaction is determined by the amount of the second antibody-bound enzyme present (bound indirectly to the solid-phase

Table 3. Cross-reactivity of commercial RIA kits[a]

Manufacturer, assay name, and assay specifics	Cross-reacting compound	% Cross-reactivity	Concentration tested (ng/mL)
Roche Abuscreen[R] RIA for Amphetamine	3,4-Methylenedioxyamphetamine	95; 17	1,000; 10,000
(High Specificity)	d, l-Amphetamine	87;17	1,000; 10,000
Calibrator: d-amphetamine	Hydroxyamphetamine HCl	35; 18	1,000; 10,000
Control range: 0-1,500 ng/mL	l-Amphetamine	27; 7.7	1,000; 10,000
Date: Nov. 1991	β-Phenylethylamine HCl	35; 18	1,000; 10,000
	Tyramine HCl	3.1; 1.5	1,000; 10,000
	3, 4-Methylenedioxymethamphetamine HCl	1.8; 0.9	1,000; 10,000
	Phenylpropanolamine HCl	1.1; 0.13	1,000; 10,000
	Methylenedioxyamphetamine[b]	158-107	100-10,000
	3, 4-Methylenedioxymethamphetamine[b]	1.6-0.26	1,000-100,000
	2-Methoxyamphetamine[b]	1.6-0.54	1,000-100,000
	2.5-Dimethoxyamphetamine[b]	1.4-0.23	1,000-100,000
	2.5-Dimethoxy-4-methylamphetamine[b]	1.4-0.12	1,000-100,000
DPC Double Antibody Amphetamine	Hydroxyamphetamine	52-29	50-1,000
Calibrator: amphetamine	Phenylethylamine	2.8	10,000
Control range: 0-1,000 ng/mL	Tyramine	2.3; 1.6	1,000-10,000
Date: Apr. 1992	3-Methoxy-3, 4-methylenedioxyamphetamine	1.4-1.1	500-10,000
	3, 4-Methylenedioxyamphetamine[c]	173-108	100-10,000
	l-Amphetamine[c]	9.2-7.6	1,000-100,000
	2, 5-Dimethoxyamphetamine[c]	4.2; 2.1	1,000-10,000
	2-Methoxyamphetamine[c]	4.1-0.66	1,000-100,000
	N-Hydroxy-3, 4-methylenedioxyamphetamine[c]	1.5-0.37	1,000-100,000
Roche Abuscreen[R] RIA for Methamphetamine	Methylenedioxymethamphetamine	108	—
(High Specificity)	l-Methamphetamine	2.8	
Calibrator: d-methamphetamine			
Control range: 0-1,500 ng/mL	l-Ephedrine[d]	<1%; ⩾0.1%	
Date: Apr. 1992	d-Pseudoephedrine[d]	<1%; ⩾0.1%	
DPC Coat-A-Count[R] Methamphetamine	Methylenedioxymethamphetamine	438; 289	100; 500
Calibrator: methamphetamine	d, l-Methamphetamine	76; 67	500; 1,000
Control range: 0-1,000 ng/mL	l-Methamphetamine	3.9-3.3	10,000-100,000
Date: Oct. 1992	Propylhexadrine	3.9-1.3	1,000-100,000
	Ranitidine	2.3; 1.2	10,000; 100,000
	l-Ephedrine	0.9	100,000
	Methylenedioxymethamphetamine[e]	>100->100	100-10,000
	Phenethylamine[e]	14-0.1	100-10,000
	N, N-Dimethylamphetamine[e]	10-3.1	100-10,000
	Amphetamine[e]	6.5-0.3	100-10,000
	Phenylephrine[e]	2.4-0.2	100-10,000
	N-Ethylamphetamine[e]	2.4-0.5	100-10,000
	Ephedrine[e]	1.7-0.5	100-10,000
	Phenmetrazine[e]	1.6-0.02	100-10,000
	Diphenhydramine[e]	1.3-0.02	100-10,000

	l-Ephedrine[d]	<1%; ⩾0.1%	
	d-Pseudoephedrine[d]	<1%; ⩾0.1%	
	Trimethobezamide[f]	<1%; ⩾0.1%	
Roche Abuscreen RIA for Barbiturates	Aprobarbital	70	—
Calibrator: secobarbital	Allylcyclopentylbarbituric acid	44	—
Control range: 0-400 ng/mL	Allylisobutylbarbituric acid	27	—
Date: Nov. 1991	Butabarbital	22	—
	Pentobarbital	21	—
	Diallylbarbituric acid	21	—
	p-Hydroxyphenobarbital	16	—
	Amobarbital	7	—
	Phenobarbital	6	—
	Barbital	3	—
DPC Coat-A-Count Barbiturates	Phenobarbital	1,820; 5,335	10; 100
Calibrator: secobarbital	Phenobarbital	182; 5,335	10; 100
Control range: 0-10,000 ng/mL	Butabarbital	256	1,000
Date: Jan. 1992	Amobarbital	228	1,000
	Pentobarbital	124; 153	1,000; 10,000
	Allylcyclopentyl barbituric acid	121; 93	1,000; 10,000
	Allobarbital	69; 67	1,000; 10,000
	Aprobarbital	39; 49	1,000; 10,000
	Butalbital	35; 25	1,000; 10,000
	Thiopental	32; 37	1,000; 10,000
	Barbital	22; 44	1,000; 10,000
	Mephobarbital	5.7-8.2	1,000-100,000
Roche Abuscreen RIA for Benzodiazepines	Diazepam[c]	284	
Calibrator: oxazepam	N-Methyloxzepam (Temazepam)	212	
Control range: 0-200 ng/mL	N-Desmethyldiazepam	202	
Date: Oct. 1990	Alprazolam	144	
	α-Hydroxyalprazolam	131	
	Pinazepam[c]	131	
	Midazolam	55	
	4-Hydroxyalprazolam	37	
	Nitrazepam	31	
	Flunitrazepam	31	
	Desmethylchlordiazepoxide	30	
	Hydroxyethylflurazepam	30	
	Medazepam[c]	30	
	Demoxepam	26	
	Halazepam[c]	25	
	Desalkylflurazepam	23	
	Clonazepate[c]	22	
	Prazepam[c]	21	
	3-Hydroxyflunitrazepam	21	
	Triazolam	20	
	Didesthylflurazepam	19	

	4-Hydroxytriazolam	13	—
	Desmethylflunitrazepam	10	—
	α-Hydroxytriazolam	5	—
	Chlorodiazepoxide[ε]	5	—
	Lorazepam	3	—
	Desmethylmedazepam	3	—
	Flurazepam	2	—
	Clonazepam	2	—
DPC Double Antibody Benzodiazepines	Alprazepam	354;330	50; 100
Calibvator: oxazepam	Tempazepam	352; 420	50; 100
Control range: 0-1,000 ng/mL	Diazepam	302; 390	50; 100
Date: Mar. 1992	Nitrazepam	96-32	50-1,000
	Demoxepam	94-47	50-1,000
	α-Hydroxyalprazepam	90-54	50-1,000
	Flunitrazepam	43-32	50-1,000
	Midazolam	21-11	50-1,000
	Desmethyldiazepam	20-58	50-1,000
	Bromazepam	16-20	50-1,000
	Chlordiazepoxide	6-15	50-1,000
	Halazepam	14-4	50-10,000
	Clorazepate	8-8	50-10,000
	Prazepam	8-3	50-10,000
	Triazolam	6-2	50-10,000
	Medazepam	4-9	50-10,000
	Lorazepam	4-1	50-10,000
	Flurazepam	3-1	50-10,000
	Clonazepam	2-1	50-10,000
Roche Abuscreen[R] RIA for Cannabinoids	11-nor-Δ^x-THC-COOH	76	—
Calibrator: 9-THC-COOH			
Control range: 0-150 ng/mL			
Date: Feb. 1992			
DPC Double Antibody Cannabinoids	11-nor-Δ^x-THC-COOH	97; 125	10; 100
Calibrator: 9-THC-COOH	11-nor-Δ^x-THC	>100; >100	100; 1,000
Control range: 0-100 ng/mL			
Date: Apr. 1993			
Immunalysis Urine Cannabinoids	11-nor-Δ^9-THC	<5	
Direct RIA Kit	11-Hydroxy-Δ^9-THC	<5	—
Calibrator: 9-THC-COOH	Cannabinol	<5	—
Control range: 0-100 ng/mL	Cannabidiol	<5	—
Date: Feb. 1993	8-β-11-Dihydroxy-Δ^9-THC	<5	—
Roche Abuscreen[R] RIA for Cocaine Metabolite	Ecgonine	2.9-0.88	1,000; 100,000
Calibrator: benzoylecgonine	Cocaine	1.2-0.72	1,000; 100,000
Control range: 0-600 ng/mL			
Date: Nov. 1991			
DPC Coat-A-Count[R] Cocaine Metabolite	Cocaine	12,700-7,952	1-1,000
Calibrator: benzoylecgonine	Cocaethylene	5,500; 1,244	100;1,000

Control range: 0-5,400 ng/mL	Ecgonine methyl ester	49	1,000
Date: Apr. 1992	Tropacocaine	28	10,000
	l-Cocaine[h]	7259	50
	l-Benzoylecgonine[h]	104	300
	l-Norcocaine[h]	64	50
	d-Cocaine[h]	7.4	5,000
	l-Ecgonine[h]	5.6	5,000
	l-Benzoylnorecgonine[h]	1.9	5,000
	l-Ecgonine methyl ester[h]	1.3	5,000
	d-Peudococaine[h]	1.0	5,000
Immunalysis Cocaine Metabolite	Cocaine	>100	—
Direct RIA Kit	Ecgonine	50	—
Calibrator: benzoylecgonine			
Control range:0-2,000 ng/mL			
Date: Sept. 1987			
DPC Coat-A-Count Fentanyl	trans-3-Methylfentanyl	32-58	5-10
Calibrator: fentanyl	*p*-Flurofentanyl	32-58	5-50
Control range: 0-7.5 ng/mL	cis-3-Methylfentanyl	4.8-27	5-10
Date: Apr.1993	Thienylfentanyl	26-16	5-50
	3-Methylfentanyl	22-15	5-50
	α-Methylfentanyl	12-5	5-50
	α-Methylthiofentanyl	7.2-9.7	5-100
	2-Hydroxyfentanyl	8.2-8.4	5-100
	Norfentanyl	1-7.6	5-100
	p-Flurofentanyl[i]	28	50
	Thienylfentanyl[i]	16	50
	3-Methylfentanyl[i]	15	50
	α-Methylfentanyl[i]	5	50
Roche Abuscreen RIA for LSD	Lysergic acid N-(methylpropyl) amide	24	
Calibrator: LSD			
Control range: 0-1 ng/mL			
Date: Apr. 1993			
DPC Coat-A-Count LSD	Lysergic acid methyl-propyl amide	5.6; 1.5	100; 1,000
Calibrator: LSD			
Control range: 0-3 ng/mL	2-Oxo-LSD[j]	11	
Date: Mar. 1992	Lysergic acid methyl-propylamide[j]	≈6	
	Lysergic acid monoethylamide[j]	≈2	
	Nor-LSD[j]	≈1	
DPC Coat-A-Count Methadone	*l*-Methadone	101-64	10-300
Calibrator: *d*-methadone	1-α-Acetyl methadol (LAAM)	50-60	200-1,000
Control range: 0-500 ng/mL			
Date: Mar. 1992			
Roche Abuscreen RIA for Methaqualone	(None listed)		
Calibrator: methaqualone			
Control range: 0-750 ng/mL			
Date: Nov. 1989			

		% Cross-reactivity	
DPC Coat-A-Count" Methaqualone Calibrator: methaqualone Control range: 0-500 ng/mL Date: Nov. 1987	4-Hydroxymethaqualone	250	100
Roche Abuscreen" RIA for Morphine Calibrator: morphine Control range: 0-600 ng/mL Date: Oct. 1991	Ethyl morphine	159	—
	Codeine	156	—
	Morphine-3-glucuronide	26	—
	Dihydrocodeine	20	—
	Hydrocodone	12	—
	Dihydromorphine	14; 7.3	1,000; 10,000
	Hydromorphone	12; 6.1	1,000; 10,000
	6-Acetylmorphine	9.0; 5.7	1,000; 10,000
	N-Norcodeine	3.4; 2.1	1,000; 10,000
	Thebaine	2.4; 1.1	1,000; 10,000
	Oxycodone	1.3;0.44	1,000; 10,000
	Dihydrocodeine^	51	10,000
	Dihydromorphine^	8.6	10,000
	Norcodeine^	4.8	10,000
	Hordenine^		
DPC Coat-A-Count" Morphine Calibrator: morphine Control range: 0-500 ng/mL Date: May 1992	Nalorphine	24-27	10-500
	Normorphine	9.0-9.1	100-10,000
	Hydromorphone	1.5-0.75	100-10,000
	Nalorphine^	22	10,000
	Dihydrocodeine^	4.7	10,000
	Normorphine^	8.7	10,000
Immunalysis Urine Heroin/Morphine Direct RIA Kit Calibrator: morphine Control range: 0-500 ng/mL Date: Sept. 1987	Morphine-3-glucuronide	75	—
	Codeine	6	—
	N-Allylnormorphine	2	—
Roche Abuscreen" RIA for Phencyclidine Calibrator: phencyclidine Control range: 0-50 ng/mL Date: Nov. 1991	1-[1-(2-Thienyl) cyclohexyl] piperidine HCl	10	1,000
	1-(1-Phenylcyclohexyl) pyrrolidine HCl	7.0	1,000
	N, N-Diethyl-1-phencyclohexylamine HCl	2.4; 0.8	1,000; 10,000
DPC Coat-A-Count" Phencyclidine Calibrator: phencyclidine Control range: 0-250 ng/mL Date: Apr. 1993	1-[1-(2-Thienyl) cyclohexyl] piperidine	95-97	10-100
	1-(1- Phenylcyclohexyl)-4-hydroxypiperidine	2.2-1.1	500-5,000
Immunalysis Phencyclidine Direct RIA Kit Calibrator: phencyclidine Control range: 0-50 ng/mL Date: Jan. 1992	1-[1-(2-Thienyl) cyclohexyl] piperidine	50	—
	1-[1-(2-Thienyl) cyclohexyl] morpholine	20	—
	1-(1-Phenylcyclohexyl) pyrrolidine	10	—
	1-(1-Phenylcyclohexyl) morpholine	8	—

"With the exceptions of those footnoted, data listed in this table are taken from the respective reagent package inserts as specified in the first column of the table. Only those compounds that show ≥ 1% cross-reactivity are listed. Compounds are listed in descending order of their reported cross-reactivities. Data listed in the ''% Cross-reactivity'' and

the "Concentration tested" columns may be separated by ";" or "—" meaning that two (for ";") or a range (for "—") of data were reported in the original literature.

[b]Data taken from Ref.(5).

[c]Data taken from Ref.(6).

[d]Data reported by Ref.(7). Using 1,000 ng/mL as the cutoff, a negative result and a positive result were observed from a control with 100,000 ng/mL and 1,000,000ng/mL, respectively, of these compounds. Thus, the cross-reactivities of these compounds are <1%, but ≥0.1%

[e]Data taken from Ref.(8).

[f]Data taken from Ref.(9). Using 1,000 ng/mL as the cutoff, a negative result and a positive result were observed from a control with 100,000 ng/mL and 1,000,000 ng/mL, respectively, of these compounds. Thus , the cross-reactivities of these compounds are <1%, but ≥0.1%.

[g]Oxazepam is a metabolite of these compounds.

[h]Data taken from Ref.(10).

[i]Data taken from Ref.(11).

[j]Data taken from Ref.(12).

[k]Data taken from Ref.(13).

[l]Data taken from Ref.(14).

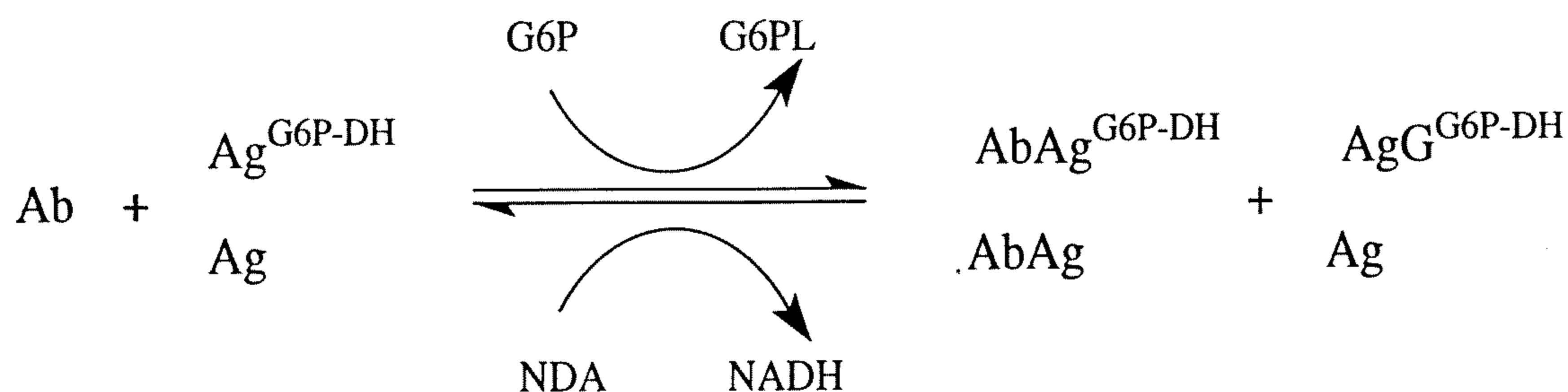Table 4. Variation of specificities of Abuscreen[®] Radioimmunoassay for Cannabinoid kits

Cross-reacting compound	Approximate % cross-reactivity[a]						
	Mar. 1987	Mar. 1988	Nov. 1988	June 1989	May 1990	Oct. 1991	Feb. 1992
11-nor-Δ^9-THC-COOH	244	76	76	76	76	49.5	76
11-Hydroxy-Δ^9-THC	38	20	20	<5	<5	2.8	<5
Δ^9-THC	5	3	3	<5	<5	<1	<5
8-β-11-Dihydroxy-Δ^9-THC	11	7	7	<5	<5	1.9	<5
8-α-Hydroxy-cannabinol	<5	13	13	<5	<5	1.4	<5
11-Hydroxy-cannabinol	<5	7	7	<5	<5	<1	<5
Cannabinol	<5	<5	<5	<5	<5	<1	<5
Cannabinol	<5	<5	<5	<5	<5	<1	<5

[a]Data taken from Abuscreen[®] Radioimmunoassay for Cannabinoid reagent package inserts dated Mar. 1987, Mar. 1988, Nov. 1988, June 1989, May 1990, and Oct. 1991.

medium). The amount of the solid-phase-bound second antibody-enzyme complex is determined by the amount of the first antibody present (bound to the analyte coated on the solid-phase midium), which is ,in turn, determined by the concentration of the analyte present in the test sample (Figure 5). Therefore, the extent of the substrate reaction is indicative of the concentration of the analyte in the test sample.

In the third application[16], the antibody is immobilized on polystyrene beads. After exposure to the test sample and a fixed amount of enzyme-labeled antigen, the polystyrene beads are washed, followed by the addition of the enzyme substrate. The substrate reaction, as determined by the amount of the bound enzyme-labeled antigen, is monitored by the absorbance change. Since the concentration of the analyte in the test sample will determine how much of the enzyme will be bound (indirectly) to the solid phase and available for catalyzing the substrate reaction, it can therefore be related to the absor-

$$Ab + \begin{matrix} Ag^{G6P\text{-}DH} \\ \\ Ag \end{matrix} \quad \overset{\displaystyle \overset{G6P \qquad G6PL}{\curvearrowright}}{\underset{\underset{NDA \qquad NADH}{\curvearrowright}}{\rightleftharpoons}} \quad \begin{matrix} AbAg^{G6P\text{-}DH} \\ \\ .AbAg \end{matrix} + \begin{matrix} AgG^{G6P\text{-}DH} \\ \\ Ag \end{matrix}$$

Figure 3. Schematic illustration of competitive immunoassay. $Ag^{G6P\text{-}DH}$: the enzyme-labeled form of the drug; Ag: the drug (analyte) to be measured; Ab: the antibody, the limiting factor in the reaction, capable of binding the drug; G6P: glucose-6 -phosphate; G6PL: 6-phosphogluconolactone; NAD: nicotinamide adenine dinucleotide.

bance change monitored.

Since the measured phenomenon is based on the activity of an enzyme capable of catalyzing multiple reactions for the conversion of an unlimited amount of reaction products, EIA can , in theory, be very sensitive. In practice, however, the amplification resulting from the accumulation of the reaction products is more than canceled out by the relatively insensitive photometric method used for the quantification of the reaction products[18].

(II). Cross-reactivities

As the most widely used immunoassay, $EMIT^R$ kits' cross-reacting characteristics are often reported by users. Some of these reports identified the exact cross-reacting compounds and quantitative cross-reactivity data, while others just reported the observed phenomena. The former category (cross-reacting compound identified) of user-reported data are listed in Table 5 along with those provided in the reagent package inserts provided by the manufacturer. Literature reports, in which the exact cross-reacting compounds are not identified, are intended in the "INTERFERENCE" section of this article.

Since the list of compiled cross-reacting compounds can never be complete, lists of com-
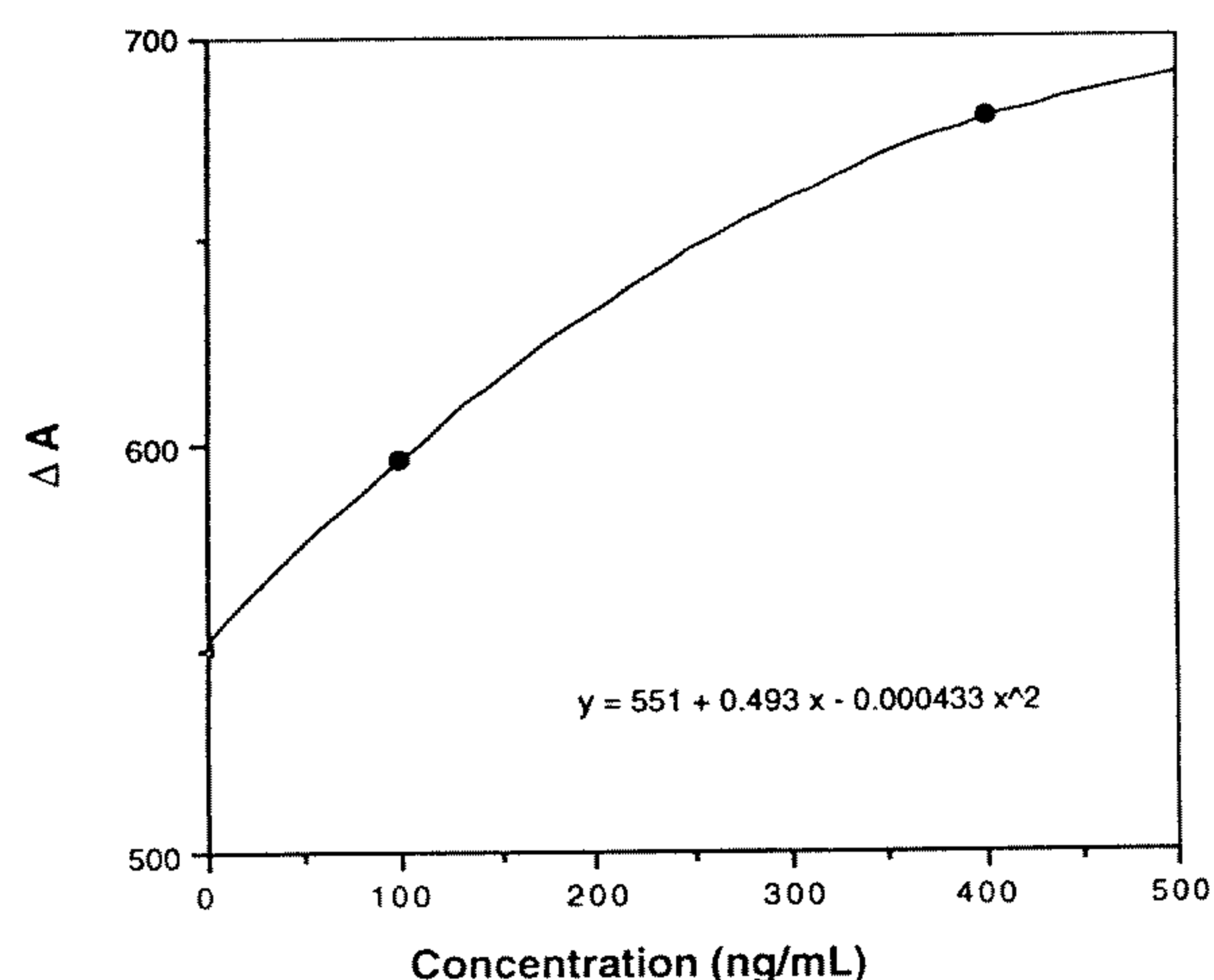
Figure 4. Example of a dose-reponse curve for $Emit^R$ d. a.u.^{TM}.

$$y = 551 + 0.493\,x - 0.000433\,x^2$$

pounds with "negative cross-reactivity"[19] are very informative and should be checked when doubt arises.

III. Fluorescence Polarization Immunoassay

(I). Basic Methodology

In parallel with the RIA and EIA technologies, the FPIA procedure is also based on the competitive binding principle of labeled antigen and unlabeled antigen, in proportion to
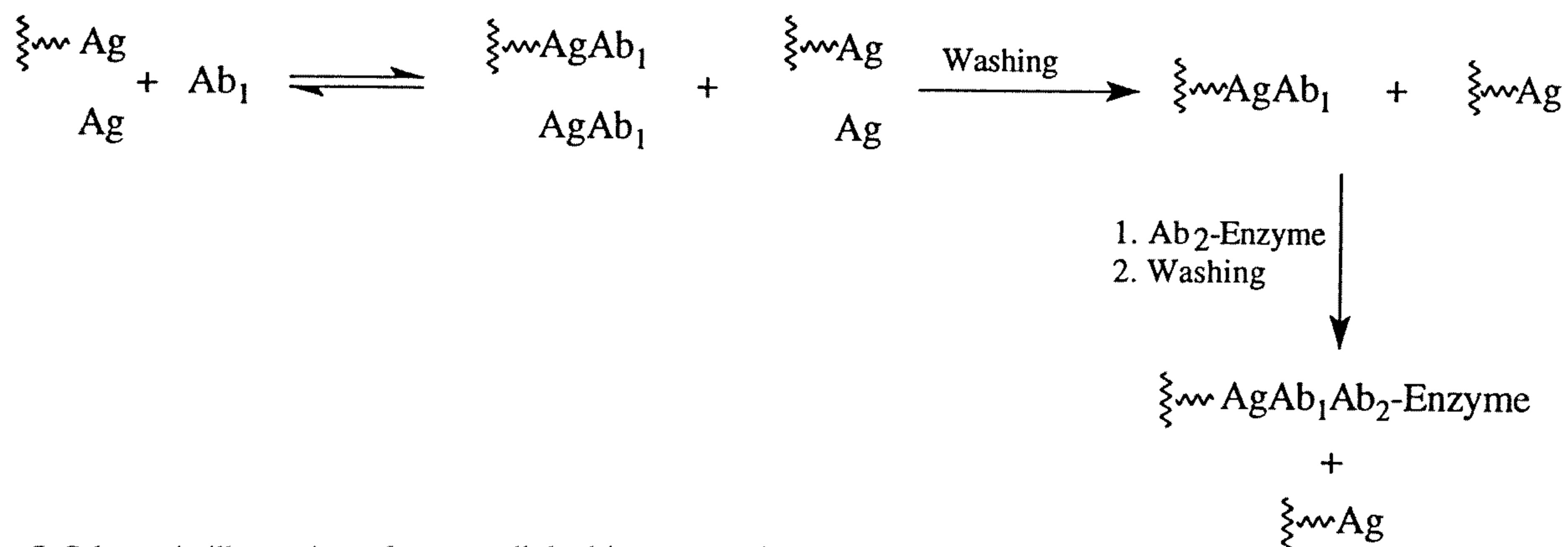
Figure 5. Schematic illustration of enzyme-linked immunosorbent assay.

their concentrations, to a limited quantity of antibody in the reaction mixture. A fluorophore is used as the label. The antibody-bound fluorophore emits at the same plane as the exciting polarized radiation, while the fluorophore on the free antigen emits at a different plane. The extent to which the labeled antigen is bound to the antibody can be monitored through the measurement of the extent of polarization. Thus, the separation step as shown for the heterogeneous RIA (second part of Figure 1) is not needed.

The fast dissociation rate constant and the stability of the antibody/fluorescein-labeled drug complex have a significant impact on the test procedure[24-28]. With the fast dissociation rate constant, the antibody and the fluorescein-labeled drug can be premixed as a single reagent. Thus, the test procedure will only involve the addition of the test sample to the premixed reagent, followed by an incubation period (to allow the displacement of a proportional amount of fluorescein-labeled drug by the analyte in the test sample) and polarization measurement. Since the premixed reagent has proven to be very stable and the reaction equilibrium, once attained, remains stable, frequent calibration of the assay is not necessary[25]. It should be noted, however, that degradation of the commercial reagent has been reported[29].

It has also been reported[24,25] that the combination of several antisera and fluorescein-

labeled antigens allow the detection of multiple drugs with a single reagent. However, since each antiserum may have different cross-reacting characteristics[25], the correlation of polarization readings with the identities and concentrations of individual analytes present may require sophisticated experimental design.

(II). *Cross-reactivity*

Fluorescence polarization immunoassay has a fundamental advantage over absorption spectrophotometric methods. Interference with the fluorescence polarization signal measurements by sample matrix is less severe. It is also advantageous over fluorescence methods since the potential intrinsic fluorescence derived from the sample matrix will not cause detection interference in polarization measurements. It has been reported, however, that interference derived from fluorescence of the sample matrix may occur if the measuring device lacks adequate optical sophistication[25]. Compounds that were reported to cross-react with TDx®/ADx®reagents are summarized in Table 6.

IV. *"Particle" Immunoassay*

In parallel with the use of radioactive isotopes, fluorescein, and enzymes, particles of appropriate size[39] can also be used as labels to serve as the basis for detecting whether a targe-

Table 5. Compounds cross-reacting to Emit® drug abuse urine assays[a]

Manufacturer, assay name, and assay specifics	Cross-reacting compound	Positive response Concentration (ng/mL)
Syva Emit® d.a.u™Amphetamine Class	d, l-Amphetamine	300
Calibrator and cutoff: d-amphetamine;	Mephentermine	400
300 ng/mL	Phentermine	400
Control range: 0-2000 ng/mL	Tranylcypromine	500
Date: Jan. 1993	Isometheptene	500
	d-Methamphetamine	1,000
	d, l-Ephedrine	1,000
	Phenmetrazine	1,000
	Phenylpropanolamine	1,000
	Nylidrin	2,000
	Isoxsuprime	6,000
	d, l-Pseudoephedrine[b]	10,000
	Pseudoephedrine[b]	15,000
Syva Emit® d.a.u™Monoclonal	d-Amphetamine	≤400
Amphetamine/Methamphetamine	Methylenedioxyamphetamine	1,000
Calibrator and cutoff: d-methamphetamine;	d, l-Amphetamine	1,000
1000 ng/mL	Methylenedioxymethamphetamine	3,000
Control range: 0-3,000 ng/mL	l-Amphetamine	10,000
Date: Nov. 1989	l-Methamphetamine	12,000
	Phentermine[b]	300
	Chloroquine[b]	3,500
	Methoxyphenamine[b]	17,000
	Ranitidine[b,c]	62,000
	N-Acetylprocainamide[b]	215,000
	Procainamide[b]	855,000
	d-Ephedrine[d]	100,000
	l-Ephedrine[d]	1,000.000
	d-Pseudoephedrine[d]	1,000,000
	l-Pseudoephedrine[d]	1,000.000
	d,l-Norephedrine[d]	1,000,000
	d,l-Norpseudoephedrine[d]	1,000,000
	Phentermine[c]	10.000
	Chloropromazine[c]	200,000
	Chloroquine[c]	200,000
	l-Ephedrine[c]	200,000
	N-Acetyl procainamide[c]	200,000
	Phenmetrazine[c]	200,000
	Phenylpropanolamine[c]	200,000
	Quinicrine[c]	200,000
	Ranitidine[c]	200,000
	Tyramine[c]	200,000
Syva Emit® II Monoclonal Amphetamine/	d-Amphetamine	1,000

Methamphetamine	*d, l*-Methamphetamine	1,200
Calibrator and cutoff: *d*-methamphetamine;	*d, l*-Amphetamine	1,500
1000 ng/mL	Benzphetamine[f]	1,500
Control range: 0-3,000 ng/mL	*l*-Methamphetamine	2,000
Date: Jan. 1993	Phentermine	2,000
	Methylenedioxyamphetamine	3,000
	l-Amphetamine	6,000
	Methylenedioxymethamphetamine	6,000
	Phenmetrazine	6,000
	Mephentermine	10,000
	Methoxyphenamine	25,000
	Fenfluramine	36,000
	Tranylcypromine	65,000
	Propranolol	160,000
	l-Ephedrine	180,000
	Tyramine	200,000
	Phenylpropanolamine	290,000
	Chloroquine	380,000
	Nor-pseudoephedrine	380,000
	Quinacrine	400,000
	Pseudoephedrine	670,000
	Selegiline[f]	
	Benzphetamine[c]	10,000
	Phentermine[c]	10,000
	l-Ephedrine[c]	200,000
	Mephentermine[c]	200,000
	Phenmetrazine[c]	200,000
Syva Emit[R] d.a.u[TM]Barbiturate	Butalbital	150
Calibrator and cutoff: secobarbital;	Aprobarbital	180
200 ng/mL	Talbutal	200
Control range: 0-1,000 ng/mL	Cyclopentobarbital	200
Date: May 1993	Alphenal	300
	Amobarbital	300
	Butabarbital	300
	Pentobarbital	300
	5-Ethyl-5-(4-hydrophenyl) barbituric acid	600
	Phenobarbital	700
	Barbital	1,000
	Thiopental	10,000
	Mephobarbital[h]	750
	Heptabarbital[b]	3,900
	Butyvinal[b]	5,000
	Allobarbital[b]	10,000
	Hexobarbital[b]	100,000
	Methohexital[b]	367,000
	p-Hydroxyphenytoin[b]	460,000

Syva Emit® II Barbiturate	Talbutal	150
Calibrator and cutoff: secobarbital;	Aprobarbital	200
200 ng/mL	Cyclopentobarbital	200
Control range: 0-1,000 ng/mL	Butabarbital	200
Date: Jan. 1993	Pentobarbital	200
	Alphenal	400
	Amobarbital	450
	Barbital	1,500
	5-Ethyl-5-(4-hydrophenyl) barbituric acid	1,500
	Phenobarbital	1,500
	Thiopental	12,000
Syva Emit® d.a.u™Benzodiazepine	Clonazepam	2,000
Calibrator and cutoff: oxazepam;	Demoxepam	2,000
300 ng/mL	Desalkylflurazepam	2,000
Control range: 0-1,000 ng/mL	N-Desmethyldiazepam	2,000
Date: Nov. 1993	Diazepam	2,000
	Flunitrazepam	2,000
	Flurazepam	2,000
	Nitrazepam	2,000
	Chlordiazepoxide	3,000
	Lorazepam	3,000
	Alprazolam[h]	100
	Prazepam[h]	100
	Medazepam[h]	145
	Halazepam[h]	155
	Triazolam[h]	170
	Clobazam[h]	230
	Temazepam[h]	260
	Lormetrazepam[h]	310
	N-l-Desalkylflurazepam[h]	322
	Bromazepam[h]	380
	Camazepam[h]	2,400
	Tetrazolam[h]	2,700
	Oxazolam[h]	4,000
	Clotiazepam[h]	4,500
	Ketazolam[h]	4,500
	Clorazepate[h]	6,150
	Midazolam[h]	185,000
	Nordiazepam[c]	500
	3-Hydroxydesalkylflurazepam[c]	1,000
	Hydroyethylflurazepam[c]	75,000
Syva Emit® II Benzodiazepine	Alprazolam	100
Calibrator and cutoff: oxazepam;	N-Desmethyldiazepam	100
200 ng/mL	Midazolam	100
Control range: 0-1,000 ng/mL	Flurazepam	110
Date: Jan. 1993	Prazepam	110

	Diazepam	110
	Triazolam	120
	α-Hydroxyalprazolam	120
	α-Hydroxytriazolam	120
	1-N-Hydroxyethylflurazepam	130
	Medazepam	130
	Halazepam	140
	Tertazepam	150
	Clobazam	180
	Temazepam	190
	Clorazepate	200
	Nitrazepam	200
	Ketazolam	210
	Flunitrazepam	220
	N-Desalkylflurazepam	230
	Lormetazepam	230
	Clonazepam	250
	Bromazepam	340
	Clotiazepam	400
	Demoxepam	500
	Norchlordiazepoxide	670
	Lorazepam	750
	Chlordiazepoxide	800
Syva Emit[R] d.a.u.™Cannabinoid 100ng Calibrator and cutoff: 9-THC-COOH; 100 ng/mL Control range: 0-200 ng/mL Date: Oct. 1992	8-β-Hydroxy-Δ^9-THC 11-Hydroxy-Δ^9-THC 11-Hydroxy-$\Delta^:$-THC 8-β-11-Hydroxy-Δ^9-THC	200 200 200 300
Syva Emit[R] II Cannabinoid 100ng Calibrator and cutoff: 9-THC-COOH; 100 ng/mL Control range: 0-200 ng/mL Date: Feb. 1993	8-β-Hydroxy-Δ^v-THC 11-Hydroxy-Δ^9-THC 11-Hydroxy-Δ^x-THC 8-β-11-Hydroxy-Δ^9-THC	200 200 200 200
Syva Emit[R] d.a.u.™Cocaine Metabolite Calibrator and cutoff: benzoylecgonine; 300 ng/mL Control range: 0-3,000 ng/mL Date: Jan. 1992	(None listed)	
Syva Emit[R] II Cocaine Metabolite Calibrator and cutoff: benzoylecgonine; 300 ng/mL Control range: 0-3,000 ng/mL Date: Jan. 1993	(None listed)	
Syva Emit[R] d.a.u.™Methadone Calibrator and cutoff: methadone; 300 ng/mL	(None listed)	

Control range: 0-1,000 ng/mL		
Date: Jan. 1992		
Syva Emit® II Methadone	(None lised)	
Calibrator and cutoff: methadone;		
300 ng/mL		
Control range: 0-1,000 ng/mL		
Date: Jan. 1993		
Syva Emit® d.a.u.™Methaqualone	Mecloqualone	1,000
Calibrator and cutoff: methaqualone;	3′-Hydroxy-methaqualone	1,000
300 ng/mL	4′-Hydroxy-methaqualone	1,000
Control range: 0-1,500 ng/mL	2′-Hydroxymethyl-methaqualone	5,000
Date: Oct. 1992		
	Mecloqualone[h]	300
	2-Methyl-3-o-(4′-hydroxy-2′-methylphenyl)-4(3H)-quinazolinone[h]	400
	2-Methyl-3-o-(3′-hydroxy-2′-methylphenyl)-4(3H)-quinazolinone[h]	500
	2-Methyl-3-o-(2′-hydroxy-2′-methylphenyl)-4(3H)-quinazolinone[h]	1,200
	2-Methyl-3-o-tolyl-3-hydroxy-4(3H)-quinazolinone[h]	1,500
	2-Methyl-3-o-tolyl-6-hydroxy-4(3H)-quinazolinone[h]	30,000
Syva Emit® II Methaqualone	Mecloqualone	300
Calibrator and cutoff: methaqualone;	4′-Hydroxy-methaqualone	300
300 ng/mL	3′-Hydroxy-methaqualone	550
Control range: 0-1,500 ng/mL	2′-Hydroxymethyl-methaqualone	2,000
Date: Jan. 1993		
Syva Emit® d.a.u.™Opiate	Codeine	1,000
Calibrator and cutoff: morphine; 300 ng/mL	Hydrocodone	1,000
Control range: 0-1,000 ng/mL	Hydromorphone	3,000
Date: June. 1992	Levorphanol	3,000
	Morphine-3-glucuronide	3,000
	Oxycodone	50,000
	Dihydrocodeine[b]	260
	Monoacetyl morphine[b]	460
	Levallorphan[b]	1,000
	Norlevorphanol[f]	23,000
	Oxymorphone HCl[f]	82,000
	Dihydrocodeine	2210,000
	Dihydromorphine	1610,000
	Levorphanol	1410,000
	Norcodeine	1010,000
Syva Emit® II Opiate	Codeine	1,000
Calibrator and cutoff: morphine;300 ng mL	Hydrocodone	1,000
Control range: 0-1,000 ng mL	Hydromorphone	3,000
Date: Jan. 1993	Levorphanol	3,000
	Morphine-3-glucuronide	3,000
	Oxycodone	50,000
Syva Emit® d.a.u.™Phencyclidine	1-(1-Phenylcyclohexyl) morpholine (PCM)	1,000
Calibrator and cutoff: phencyclidine;	1-(1-Phenylcyclohexyl) pyrrolidine (PCPy)	1,000

75 ng/mL	1-[(1-(2-Thienyl)-cyclohexyl] piperidine (TCP)	1,000
Control range: 0-400 ng/mL	1-[(1-(2-Thienyl)-cyclohexyl] pyrrolidine (TCPy)	1,000
Date: May 1988	4-Phenyl-4-piperidinocyclohexanol	2,000
	N, N-Diethyl-1-phenylcyclohexylamine (PCDE)	3,000
	1-(4-Hydroxypiperidino) phenylcyclohexane	3,000
	1-[1-(2-Thienyl)-cyclohexyl] morpholine (TCM)	5,000
Syva Emit[R] II Phencyclidine	1-(1-Phenylcyclohexyl) morpholine (PCM)	1,000
Calibrator and cutoff: phencyclidine	1-(1-Phenylcyclohexyl) pyrrolidine (PCPy)	1,000
25 ng/mL	1[1-(2-Thienyl)-cyclohexyl] piperidine (TCP)	1,000
Control range: 0-75 ng/mL	1[1-(2-Thienyl)-cyclohexyl] pyrrolidine (TCPy)	1,000
Date: Dec. 1992	4-Phenyl-4-piperidinocyclohexanol	2,000
	N, N-Diethyl-1-phenylcyclohexylamine (PCDE)	3,000
	1-(4-Hydroxypiperidino) phenylcyclohexane	3,000
	1-[1-(2-Thienyl)-cyclohexyl] morpholine (TCM)	5,000
Syva Emit[R] d.a.u.[TM]Propoxyphene	Norpropoxyphene	10,000
Calibrator and cutoff: propoxyphene;		
300 ng/mL		
Control range: 0-1,000 ng/mL		
Date: Sept. 1992		
Syva Emit[R] II Propoxyphene	Norpropoxyphene	4,200
Calibrator and cutoff: propoxyphene;		
300 ng/mL		
Control range: 0-1,000 ng/mL		
Date: Dec. 1993		

"With the exceptions of those footnoted, data listed in this table are taken from the respective reagent package inserts as specified in the first column of the table. When present at the concentration level listed in the "Positive response concentration" column, the crossing-reacting compound show an equal or greater response than the calibrator at the cutoff concentration.

*These compounds are listed in Ref. [19] as cross-reacting compounds and show positive result if present at or higher than the concentrations listed in the "Positive response concentration" column.

Ref. [20] reported that approximately 100,000 ng/mL of ranitidine generated an absorbance change equivalent to 1,000 ng/mL of d-methamphetamine. This study also reported that ranitidine did not interfere with the EMIT[R] d.a.u.[TM]polyclonal amphetamine assay.

Data taken from Ref.(7).

Data taken from Ref.(21).

Amphetamine and methamphetamine are metabolites of these drugs.

Data taken from Ref.(22).

Data taken from Ref.(23).

Data taken from Ref.(13).

ted antibody-antigen reaction has occurred. Thus, latex particles were utilized for the development of immunoassay test methodology for morphine[40], barbiturates[41], and methamphe-tamine[42,43].

The principle of this methodology is based on the competitive binding of latex particle-labeled drugs with the analyte, if present, in the test

Table 6. Compounds cross-reacting to Abbott TDx®/ADx® assays[a]

Manufacturer, assay name, and assay specifics	Compound	% Cross-reactivity	Concentration tested (ng/mL)
Abbott TDx® Amphetamine/	Propylhexedrine	108-14	250-10,000
Methamphetamine	p-Hydroxyamphetamine	102; 103	500;1,000
Calibrator:d, l-amphetamine	d, l-Methamphetamine	93-42	150-3,000
Control range: 0-3,000 ng/mL	d-Methamphetamine	93-39	150-3,000
Date: 12/01/1987	d-Amphetamine	80-59	150-3,000
	l-Amphetamine	80-33	150-3,000
	Fenfluramine	14-1.6	2,500-100,000
	l-Methamphetamine	8.7; 7.3	1,500; 3,000
	3, 4-Methylenedioxyethylamphetamine,	30-2.5	5,000-50,000
	Phenmetrazine	12-0.59	1,000-100,000
	Phentermine	9.8; 8.0	5,000; 10,000
	Mephentermine	8.4-2.5	5,000-50,000
	Tyramine	2.6-2.3	5,000-100,000
	Labetalol	2.4-1.2	10,000-250,000
	Phenethylamine	2.2; 2.5	50,000; 100,000
	Ranitidine	1.2-0.03	10,000-1,000,000
	Isoxsuprine	1.1-0.64	10,000-100,000
	Fenfluramine[b]	14	10,000
	Phentermine[b]	5	10,000
	Phenmetrazine[b]	4	10,000
	Phenethyamine[b]	2	10,000
	3, 4-Methylenedioxymethamphetamine[c]	118-18	150-10,000
	3, 4-Methylenedioxyethylamphetamine[c]	47-12	150-10,000
	3, 4-Methylenedioxyamphetamine[d]	465-503	1000-10,000
	Ritodrine[c]	0.6-1.4	125,000-350,000
	d-Methamphetamine[e]	207-66	200-5,000
	d-Amphetamine[e]	116-53	200-10,000
	3, 4-Methyienedioxyethylamphetamine[e]	73-4.5	200-100,000
	l-Methamphetamine[e]	70-116	200-2,000
	l-Amphetamine[e]	66-58	200-5,000
	3, 4-Methylenedioxymethamphetamine[e]	47-7.4	200-10,000
	4-Hydromethamphetamine[e]	27-4.7	200-5,000
	3, 4-Methylenedioxyamphetamine[e]	26-4.8	200-10,000
	Ephedrine[e]	26-3.9	1,000-100,000
	Phenylpropanolamine[e]	13-7.4	1,000-50,000
Abbott ADx® Amphetamine/	d, l-Amphetamine	80-217	150-3,000
Methamphetamine II	4-Chloroamphetamine	73-124	300-5,000
Calibrator:d-amphetamine	d-Methamphetamine	100-60	150-8,000
Control range: 150-4,000 ng/mL	3, 4-Methylenedioxyethylamphetamine	70-96	300-3,000
Date: 1992	3, 4-Methylenedioxymethamphetamine	63-51	300-8,000
	d, l-Methamphetamine	57-43	300-8,000

	3, 4-Methylenedioxy-N-ethylamphetamine	47-17	300-8000
	l-Amphetamine	37-29	300-8,000
	Propylhexedrine	34-19	1,000-10,000
	p-Hydroxyamphetamine	27-31	1,000-10,000
	Fenfluramine	13-5.5	1,000-50,000
	Isometheptene	11-10	2,000-50,000
	Mephentermine	4.7-5.3	10,000-100,000
	l-Methamphetamine	4.3-5.1	3,000-8,000
	4-Methyl-2, 5-dimethoxyamphetamine	4.7-2.4	3,000-100,000
	4-Ethyl-2, 5-dimethoxyamphetamine	4.7-1.9	3,000-100,000
	Phenethylamine	2.4-3.1	10,000-100,000
	Methoxyphenamine	2.0-2.4	10,000-100,000
	d, l-Amphetamine[g]	120-210	200-2,000
	3, 4-Methylenedioxyamphetamine[g]	136-170	200-2,000
	d-Methamphetamine[g]	108-86	200-5,000
	3, 4-Methylenedioxymethamphetamine[g]	92-104	200-5,000
	4-Hydromethamphetamine[g]	79-73	500-10,000
	3, 4-Methylenedioxyethylamphetamine[g]	67-31	200-10,000
	l-Amphetamine[g]	61-66	200-10,000
	d, l-Methamphetamine[g]	58-66	200-10,000
	2-Methoxyamphetamine[g]	27-35	500-10,000
	l-Methamphetamine[g]	7.2-10	1,000-10,000
	2, 5-Dimethoxyamphetamine[g]	6.5-4.1	5,000-50,000
	Phentermine[h]		10,000
	Mephentermine[h]		200,000
	Phenmetrazine[h]		200,000
	Phenylpropanolamine[h]		200,000
	Tyramine[h]		200,000
Abbott TDx[R] Barbiturates	Butobarbital	160-129	200-1,200
Calibrator: secobarbital	Amobarbital	155-133	200-1,200
Control range: 0-2,000 ng/mL	Phenobarbital	105-155	105-1,200
Date: 12/01/1987	Cyclopentobarbital	145-115	200-1,200
	Alphenal	110-137	200-1,200
	Butalbital	100-101	200-2,000
	Pentobarbital	105-72	200-2,000
	Brallobarbital	90-91	200-2,000
	Tallbutal	90-84	200-2,000
	Butabarbital	85-60	200-2,000
	5-Ethyl-5-(4-hydroxyphenyl)-barbituric acid	60-54	200-2,000
	Aprobarbital	45-36	200-2,000
	Allobarbital	25-29	200-2,000
	p-Hydroxyphenytoin	3.4	10,000
	Glutethimide	1.8	10,000
	Phenytoin	1.0-1.0	1,000-100,000
	Barbital	1.0	2,000
	Glutethimide	15	2,000

	Phenytoin[f]	10	2000
	Primidone[f]	2	2000
	Phenytoin[f]		
	5-(p-Hydroxyphenyl)-5-phenylhydantoin[f]		
Abbott ADx® Barbiturates II U	Cyclopentobarbital	868	200
Calibrator: secobarbital	Tallbutal	266-250	200-700
Control range: 150-4,000 ng/mL	Butabarbital	245-236	200-1,200
Date: 1992	Cyclopentobarbital	145-115	200-1,200
	Butalbital	114-106	200-1,200
	Alphenal	108-82	200-2,000
	Brallobarbital	94-83	200-2,000
	Cyclobarbital	76-70	200-2,000
	Phenobarbital	71-51	200-2,000
	Pentobarbital	65-67	200-2,000
	Aprobarbital	62-65	200-2,000
	Metharbital	53-47	200-2,000
	Butobarbital	46-52	200-2,000
	Amobarbital	36-34	200-2,000
	Allobarbital	33-29	200-2,000
	Thiopental	12-7.0	400-2,000
	5-Ethyl-5-(4-hydroxyphenyl)-barbituric acid	9.7-6.4	700-2,000
	Glutethimide	9.0-4.8	1,000-10,000
	Barbital	6.4-6.0	1,200-2000
Abbott ADx® Benzodiazepines	Diazepam	123-144	200-2,400
Calibrator: nordiazepam	Prazepam	119-70	200-2,400
Control range: 0-2,400 ng/mL	Alprazolam	117-61	200-2,400
Date: 1992	Medazepam	99-47	200-2,400
	1-N-Hydroxyethylflurazepam	90-52	200-2,400
	Midazolam HCl	90-45	200-2,400
	Nimetazepam	90-18	100-10,000
	Nitrazepam	89-31	200-2,400
	Triazolam	83-23	200-2,400
	Oxazepam	76-36	200-2,400
	Flurazepam	75-27	200-2,400
	Temazepam	74-48	200-2,400
	Flunitrazepam	70-31	200-2,400
	Desalkylflurazepam	59-37	200-2,400
	Lorazepam	50-17	200-2,400
	Clonazepam	48-15	200-2,400
	Bromazepam	41-14	200-2,400
	Demoxepam	34-13	200-2,400
	Clobazam	27-8.1	1,000-10,000
	Chlordiazepoxide	23-6.7	200-2,400
	Norchlordiazepoxide	22-7.4	200-2,400
Abbott TDx® Cannabinoids	8-β-11-di-Hydroxy-Δ⁹-THC	119-39	25-200
Calibrator: 8-THC-COOH	8-β-Hydroxy-Δ⁹-THC	99-37	25-200

Control range: 0-150 ng/mL	9-THC-COOH	94-70	25-200
Date: 12/01/87	11-Hydroxy-Δ^9-THC	60-44	25-200
	Cannabinol	26-20	25-200
Abbott ADx® Cannabinoids	11-Nor-Δ^8-THC-9-COOH	111-109	25-100
Calibrator: 9-THC-COOH	8-β-11-Hydroxy-Δ^9-THC	108-29	25-200
Control range: 0-135 ng/mL	8-β-11-Dihydroxy-Δ^9-THC	107-29	25-200
Date: 1992	11-Hydroxy-Δ^9-THC	72-60	25-200
	Cannabinol	56-35	25-200
Abbott ADx® Cocaine Metabolite	Cocaine	0.9; 1.0	10,000; 100,000
Calibrator: benzoylecgonine			
Control range: 0-5,000 ng/mL	Ecgonine metyl ester^k	1.2	10,000
Date: 1992			
Abbott ADx® Methadone	l-α-Methadol	56-20	250-4,000
Calibrator:benzoylecgonine	l-α-Acetylmethadol	26-11	500-4,000
Control range: 0-4,000 ng/mL	d-β-Acetylmethadol	14-7.8	1,000-4,000
Date: 1992	l-β-Acetylmethadol	13-6.5	1,000-4,000
	d-α-Methadol	4.5	4,000
	l-α-Acetyl-N-normethadol	2.8	4,000
Abbott ADx® Opiates	Codeine	120-114	50-500
Calibrator: morphine	Hydrocodone	120-47	50-1,000
Control range: 0-1,000 ng/mL	Hydromorphone	114-37	50-1,000
Date: 1992	Dihydromorphine	108-47	50-1,000
	6-Monoacetylmorphine	96-45	50-1,000
	Levorphanol	79-7.9	100-10,000
	Ethylmorphine	77-95	200-1,000
	Dihydrocodeine	68-53	200-1,000
	Diacetylmorphine	69-40	200-1,000
	Thebaine (dimethylmorphine)	63-6.9	100-10,000
	Morphine-3β-D-glucuronide	58-36	50-1,000
	Levallorphan	36-0.3	100-100,000
	Promethazine	35-0.1	100-100,000
	Oxycodone	24-0.5	200-100,000
	Oxymorphone	18-0.4	200-100,000
	Nalorphine	14-0.7	1,000-100,000
	N-Norcodeine	6.7-0.5	1,000-100,000
	Cyclazocine	4.1-0.8	1,000-10,000
	N-Normorphine	4.1-0.3	1,000-100,000
	Meperidine	3.6-0.1	1,000-250,000
	Alphaprodine	3.4-0.2	1,000-100,000
	Naloxone	3.3-0.2	1,000-100,000
	Naltrexone	2.7-0.1	1,000-100,000
	Dihydrocodeine^l	67	10,000
	Dihydromorphine^l	46	10,000
	Levorphanol^l	8.1	10,000
	Nalorphine^l	3.3	10,000
	Norcodeine^l	2.8	10,000

	Normorphine[i]	1.2	10,000
Abbott TDx® Phencyclidine	4-Hydroxypiperidine PCP	46; 38	100; 1,000
Calibrator: phencyclidine	Levallorphan	1.6-0.18	100-100,000
Control range: 0-500 ng/mL			
Date: 12/01/1987			
Abbott ADx® Phencyclidine II	1-[1-(2-Thienyl) cyclohexyl] piperidine	59-47	25-1,000
Calibrator: phencyclidine	4-OH pip phencyclidine	22-15	25-1,000
Control range: 0-500 ng/mL			
Date: 1992			
Abbott ADx® Propoxyphene	N-Norpropoxyohene	80-30	200-1,500
Calibrator: phencyclidine			
Control range: 0-1,500 ng/mL	Norpropoxyohene[m]	93-29	100-1,500
Date: 1992			
Abbott TDx® Tricyclic	Amitriptyline	101-109	
Antidepressants	Desipramine	99-92	100-500
Calibrator: imipramine	Nortriptyline	95-86	100-500
Control range: 0-1,000 ng/mL	Trimipramine	93-70	100-500
Date: 1992	Protriptyline	91-78	100-500
	Norclomipramine	61	500
	Cyclobenzaprine	55	300-1,000
	Clomipramine	51-41	100-500
	Nordoxepin	44	500
	Doxepin	39-28	100-500
	Dothiepin	39-28	100-500
	Cyproheptadine	33	1,000
	Perphenazine	28-23	300-1,000
	2-Hydroxydesipramine	18-11	300-1,000
	Promethazine	16-13	300-1,000
	Maprotiline	14	1,000
	2-Hydroxyimipramine	13-8	300-1,000
	cis-10-Hydroxyamitriptyline	9	300
	Orphenadrine	6.5-0.6	1,000-100,000
	trans-10-Hydroxyamitriptyline	6	300
	cis-10-Hydroxynortriptyline	6	300
	trans-10-Hydroxynortriptyline	6	300
	Chlorpromazine	6	300-1,000
	Diphenhydramine	5.4-0.5	1,000-100,000
	Mianserin	5.2	1,000
	Thioridazine	2.8-0.4	1,000-100,000
	Amoxapine	2	1,000
	Trazodone	1	1,000
ADx® Tricyclic antidepressants	Nortriptyline	97-81	100-500
Calibrator: imipramine	Imipramine N-oxide	89-92	75-1,000
Control range: 0-1,500 ng/mL	Amitriptyline	80-91	100-500
Date: 1992	Desipramine	90-87	100-500
	Dothiepin	69-59	100-500

Trimipramine	67-55	100-500
Protriptyline	63-54	100-500
Cyclobenzaprine	53-43	300-1,000
Clomipramine	51-41	100-500
Doxepin	42-32	100-500
Cyproheptadine	30	1,000
Prochlorperazine	30-18	100-1,000
Perphenazine	28-23	300-1,000
Nordoxepin	27	500
Chlorpromazine	20-14	300-1,000
2-Hydroxydesipramine	18-12	300-1,000
2-Hydroxyimipramine	16-9.7	300-1,000
cis-10-Hydroxyamitriptyline	13	300
Promethazine	13-9.0	300-1,000
cis-10-Nortriptyline	8.7	300
Maprotiline	8.2	1,000
Mianserin	5.2	1,000
Thioridazine	2.8-0.4	1,000-100,000
Pimozide	2.7-0.3	1,000-100,000
Orphenadrine	2.6-0.5	1,000-100,000
Amoxapine	2.0	1,000

[a]With the exceptions of those footnoted, data listed in this table are taken from the respective reagent package inserts as specified in the first column of the table. Only those compounds that show $\geq$ 1% cross-reactivity are listed. Compounds are listed in descending oreder of their reported cross-reactivities. Date listed in the "% Cross-reactivity" and the "Concentration tested" columns may be separated by ";" or "—" meaning that two (for ";") or a range (for "—") of data were reported in the original literature.

[b]Data taken form Ref.(30).

[c]Data taken form Ref.(31).

[d]Data taken form Ref.(32).

[e]Data taken form Ref.(33).

[f]Data taken form Ref.(34) using Amphetamine Class reagent.

[g]Data taken form Ref.(34) using Amphetamine/Methamphetamine II reagent.

[h]Data taken form Ref.(21). Positive results were observed with the levels of the compounds tested.

[i]Data taken form Ref.(35).

[j]Data taken form Ref.(36).

[k]Data taken form Ref.(37).

[l]Data taken form Ref.(13).

[m]Data taken form Ref.(38).

sample for a limited amount of antibody available. For a negative sample, the antibody will cross-link to sufficient latex particle-labeled drug molecules to produce agglutination particles that are large enough for visual detection. Thus, a negative sample will result in the occurrence of agglutination, while a highly positive sample will result in a smooth milky appearance of the original reaction medium.

This approach has recently been commercialized by Roche Diagnostic Systems and convenient Abuscreen®Ontrak®assay kits are avail-

able for common drugs of abuse. Since visual inspection is the basis of detection, the differentiation of samples containing the analyte at or near the "cutoff" level will always be somewhat subjective. While this line of products may be useful for field applications, an objective detecting mechanism and automation process will be helpful for applications in a high-volume test environment.

Indeed, the same manufacturer marketed a different line of product (Abuscreen® Online™) in which mechanisms for objective detection and automation are featured. Working under the same principle, this test methodology utilizes a microparticle label and a photometric detection device[44]. Based on the competitive binding principle used for all immunoassays addressed earlier, the underlying aggregation reaction will proceed when the analyte is absent in the test sample. Under this circumstance, lower light transmission will reach the photometric detector.

Since this is a relatively new product, independent literature data are generally lacking. The cross-reactivity data as provided in the product package inserts for the currently available assay kits are shown in Table 7.

INTERFERENCE

Interference can be broadly defined as the observation of a test result that does not provide the intended diagnostic finding reflecting the true status of the specimen. The most widely studied interferences are the "false" positive responses (on the initial test) resulting from the presence of cross-reacting compounds listed in Tables 3 and 5-7. (These positive initial tests are then eliminated by GC/MS procedures.)

In addition to the interference caused cross-reacting compounds, the following sample conditions may also generate a test result leading to an incorrect interpretation of the sample status: (a) presence of the targeted analyte derived from sources other than the targeted drugs of abuse, (b) the presence of cross-reacting compounds with unknown structure, (c) specimen conditions

that cause non-specific binding, and (d) specimen conditions that interfere with the assay's detection mechanism.

One area that has attracted much attention in the drug testing communities is the responses of various immunoassays toward the intentional addition of "adulterants". Some of these adulterants may actually destroy the targeted drugs, thus rendering the specimen "truly negative". Under this circumstance, immunoassays (and other test methodologies) are expected to respond negatively. Other adulterants may, however, cause non-specific binding or create interference on the detection mechanism. A reliable immunoassay should use an antibodies with desired specificities and a detection mechanism that is robust toward interfering conditions.

I. *Presence of Targeted Analytes Derived from Unintended Exposure, Food Consumption, and Licit Medication*

Some of the analytes targeted as the indicators of drug abuse may derived from unintended exposure, food consumption, or licit medication. Low quantities of marijuana and cocaine related metabolites have been detected in individuals who were subjected to passive inhalation [45] or skin absorption[46].

It is well known[47] that morphine and codeine may be observed in urine samples collected from individuals consuming poppy seed-containing food items or morphine/codeine-containing prescriptions. Methamphetamine detection may also be caused by using Vicks Nasal Inhaler[R(48)] and other medication. Methamphetamine and amphetamine have been reported as the metabolites of a substantial number of licit drugs. A list recently compiled[49] by a drug urinalysis expert included Amphetaminil, Benzphetamine, Clobenzorex, Deprenyl, Dimethylamphetamine, Ethylamphetamine, Famprofazone, Fencamine, Fenethylline, Fenproporex, Furfenorex, Mefenorex, Prenylamine, Mesocarb. It is thus obvious that test results needed to be interpreted carefully by those with thorough

Table 7. Compounds cross-reacting to Abuscreen® Online™assays[a]

Manufacturer, assay name, and assay specifics[b]	Cross-reacting compound	% Cross-reactivity
Roche Abuscreen® Online™for Amphetamines	*d, l*-Amphetamine	51
Targeted drug: amphetamine and methamphetamine	Methylenedeoxyamphetamine	32
and their metabolites	*p*-Hydroxyamphetamine	14
Date: Apr. 1992	*l*-Amphetamine	2
	β-Phenethylamine	2
Roche Abuscreen® Online™for Barbiturates	Cyclopentobarbital	95
Targeted drug: barbiturates	Aprobarbital	68
Date: Feb. 1992	Allobarbital	61
	Butabarbital	41
	Butalbital	40
	Pentobarbital	35
	Phenobarbital	32
	Amobarbital	28
	p-Hydroxyphenobarbital	27
	Barbital	21
Roche Abuscreen® Online™for Benzodiazepines	Alprazolam	96
Targeted drug: benzodiazepines	(α-Hydroxyalprazolam)[c]	112
Date: Sept. 1992	(4-Hydroxyalprazolam)	146
	Bromazepam	75
	Chlordiazepoxide	55
	(Desmethylchlordiazepoxide)	60
	Clonazepam	56
	Clorazepate K⁺salt	43
	Demoxepam	96
	Diazepam	105
	(Oxazepam)	98
	(*N*-Methyloxazepam)	95
	Flunitrazepam	52
	(Desmethylflunitrazepam)	56
	(3-Hydroxyflunitrazepam)	24
	Flurazepam	61
	(Desalkyflurazepam)	49
	(Didesethylflurazepam)	84
	(Hydroxyethylflurazepam)	88
	Lorazepam	59
	Medazepam	40
	(Desmethylmedazepam)	38
	Midazolam	96
	Nitrazepam	81
	(7-Aminonitrazepam)	52
	Pinazepam	106
	Prazepam	84

	Triazolam	96
	(α-Hydroxytriazolam)	98
	(4-Hydroxytriazolam)	52
Roche Abuscreen® Online™for Cannabinoids		
Targeted drug: cannabinoids	8-α-Hydroxy-Δ⁹-THC	22
Date: Sept. 1992	11-Hydroxy-Δ⁹-THC	18
	Δ⁹-THC	11
	8-β-11-Dihydroxy-Δ⁹-THC	10
	11-Hydroxycannabinol	5
	cannabinol	2
Roche Abuscreen® Online™for Cocaine Metabolite	Ecgonine	2.3
Targeted drug: benzoylecgonine		
Date: Jan. 1991		
Roche Abuscreen® Online™for Methadone	Methadol	120
Targeted drug: methadone	Hydroxymethadone	52
Control range: 0-600	L-α-Acetylmethadol HCl	30
Date: May 1993	Promethazine	2.5
Roche Abuscreen® Online™for Opiates	Codeine	199
Targeted drug: morphine and its metabolites	Dihydromorphine	178
Date: Jan. 1991	6-Acetylmorphine	80
	Thebaine	79
	Hydrocodone	77
	Dihydromorphine	73
	Hydromorphone	73
	Morphine 3-glucuronide	62
	Ethyl morphine	39
	Oxycodone	6
	Meperidine	3
	N-Norcodeine	2
Roche Abuscreen® Online™for Phencyclidine	Thienylcyclohexylpiperidine	64
Targeted drug: phencyclidine and its metabolites		
Date: Apr. 1992		

ᵃWith the exceptions of those footnoted, data listed in this table are taken from the respective reagent package inserts as specified in the first column of the table. Only those compounds that show ≥ 1% cross-reactivity are listed. Compounds are listed in descending order of their reported cross-reactivities.

ᵇTargeted drugs are those listed in the Intended Use sections of the package inserts.

ᶜCompounds listed with indentation and inside parentheses are metabolites of the preceding drugs.

knowledge on the subject area.

II. Unknown Cross-reacting Compound and Non-specific Binding

It has been reported that unknown meta-bolite(s) of chlorpromazine[50], brompheniramine [50], and labetalol[51] caused EMIT® d.a.u. ™Monoclonal Amphetamine/Methamphetamine Assay to general false positive results. Metabolites, not the parent drugs, were believed to be the responsible cross-reacting compounds be-

cause: (a) these drugs were prescribed for the urine specimen donors; (b) these parent drugs were present in the urine specimens, and (c) studies on control samples with various concentrations of the parent drugs alone failed to generate a positive result. Since reference metabolites of these drugs are not available, the exact cross-reacting metabolites cannot be identified.

Urine specimens from patients using pipothiazine or fluspirilene prescriptions also generated false positive results when tested by the EMIT® d.a.u.™ Monoclonal Amphetamine/Methamphetamine Assay[52]. Since no study was conducted on the parent drugs alone, it is not known whether the parent drugs or their metabolites caused the false results. Other interferences reported include (a) false EMIT® positives for amphetamine by benzathine[53], (b) false EMIT® positives for cannabinoids by the acute dose of ibuprofen and (c) chronic dose of naproxyn[54], and false TDx® positives for barbiturates by chronic dose of ibuprofen and naproxyn[54]. The false positives from the cannabinoids and barbiturates studies[54] were observed only from a very small fractions of specimen studied-the vast majority of specimens studied did not generate false positive result.

False EMIT® negatives of cannabinoid assays[55,56] and other drugs[57] have also been reported. In one study, an alarming six false negatives (out of 41 patient samples tested) were reported[56]. Improved performance has been reported[58] with the new calibration formulation that does not use a surfactant and uses 9-THC-COOH (instead of 11-nor-Δ^8-THC-COOH) as the calibrator.

III. Detection Mechanism

The causes for many observed false negative results are often unknown. Based on the observation that the average absorbance change of completely negative postmortem urine samples is lower than that generated by samples collected from healthy persons, it has been postulated[59] that some inhibitors to the EMIT® reactions may be present in the postmortem urine samples. It is possible that the observed lower absorbance changes are due to the presence of nonspecific interacting materials that cause a higher initial absorbance value. Indeed, it has been reported[60] that many postmortem urine specimens had absorbance change values lower than those produced by the negative calibrators.

Enzyme immunoassay also suffers a potential spectrometric interference caused by substances that are present in the sample. For example, it was reported that:

1. p-nitrophenol, a metabolite of methyl parathion, can absorb strongly in the 340 nm region at pH 8.0 and thus cause interference[61];

2. the presence of metronidazole[62] or mefenamic acid[64] cause excessively high initial absorbance values, thus preventing the assessment of EMIT® test data.

An interesting study reported[64] that the addition of excess reagent antibody will cause EMIT® to result in false negative for samples containing benzoylecgonine. It was reasoned that when excess antibody is added, the mount of enzyme-labeled drug bound by the antibody is increased. This results in a greater amount of enzyme being inhibited by the antibody. The resulting decreased signal (decreased conversion of NAD to NADH) would decrease the sensitivity of the EMIT® assay to the drug in the urine near the threshold concentration in urine. When the total antibody concentration approaches the total amount of drug (the sum of enzyme-labeled and nonenzyme-labeled species), the amount of free enzyme-labeled drug decreases toward zero, and the EMIT® absorbance signal is markedly decreased. The use of a "high tech" adulterant that tampers with the underlying assay detection mechanism is intellectually challenging and can only be used by those who have ready access to the specific antibody.

IV. Adulterants

Studies conducted in several drug testing laboratories revealed that the presence of common

accessible adulterants do affect the responses of common immunoassays. The effects of adulterants vary with the drug categories tested and the immunoassay methodologies used. Adulterants that were reported to cause significant interferences have been reviewed recently[65]. Information included in this review and newer data are summarized in Table 8.

Most of the studies included in Table 8 did not compare the effects of the adulterants on various immunoassays under the same conditions; it is therefore difficult to make general statements concerning the robustness of one methodology over the others. It seems to be clear, however, that cannabinoid assays are most susceptible to the influence of adulterants.

Numerous mechanisms have been proposed to account for the observed interference[66,68-70], the exact cause of these interferences are generally unknown. It has been proved, however, that

Table 8. Effects of adulterants on immunoassays

Substance	Amount	Method	Drug category[a]	Sample[b]	Effect[c]	Ref.
Ammonia	5%, 10%	RIA	COC	–	—	[66]
	5%, 10%, 14%	RIA	THC	+/–	+++	[66,67]
	14%	RIA	PCP	+/–	+++/++	[67]
	10%	FPIA	BAR	+/–	+++/++	[68]
	14%	FPIA	PCP	+	—	[67]
	14%	FPIA	THC	+/–	+++/+	[67]
Potassium hydroxide	0.5M, 5M	RIA	THC	+/–	++++	[67]
	0.5M, 5M	RIA	PCP	+/–	++++	[67]
	5M	FPIA	THC	+/–	+++	[67]
	0.5M, 5M	FPIA	PCP	+	—	[67]
Sodium bicarbonate	40 mg/mL	EMIT[d]	BAR	+	++	[69]
	40 mg/mL	EMIT[d]	PCP	+	—	[69]
	40 mg/mL	RIA	AMP, BAR, THC	+/–	++	[69]
	40 mg/mL	FPIA	PCP	+	—	[69]
Liquid Bleach[e]	12 μL/mL	EMIT[d]	THC	+	—	[70]
	23 μL/mL	EMIT[d]	AMP, BAR, OPI	+	—	[70]
	42 μL/mL	EMIT[d]	COC	+	—	[70]
	125 μL/mL	EMIT[d]	BEN	+	—	[70]
	10%	RIA	AMP	+	—	[66]
	10%, 5%	RIA	THC, OPI	+	—	[66]
	50%	RIA	PCP	+	—	[67]
	5%	RIA	THC	–	++	[67]
	50%	RIA	THC	+/–	++++	[67]
	5%, 50%, 10%	FPIA	THC	+	—[f]	[67,68]
	10%	FPIA	OPI	+	—	[68]
	1 drop/10 mL	EMIT[d]	THC	+	–[g]	[71]
	10.5 μL/mL	EMIT[d]	AMP, OPI, PCP, THC, BEN	+	—	[69]
	10.5 μL/mL	RIA	AMP, OPI, PCP	+	—	[69]
	10.5 μL/mL	RIA	BAR	+	++	[69]
	10.5 μL/mL	FPIA	AMP, OPI, PCP, THC	+	—	[69]
	10.5 μL/mL	FPIA	BEN	–	+++	[69]

Drano® (NaOH + NaHClO₄)	12 µL/mL	EMIT®	THC	+	——	[70]
	23 µL/mL	EMIT®	AMP, BAR, OPI	+	——	[70]
	42 µL/mL	EMIT®	COC	+	——	[70]
	125 µL/mL	EMIT®	BEN	+	——	[70]
	10%	RIA	AMP, BAR, COC, OPI, PCP, THC	+/−	++++	[66]
	1%	RIA	COC	+	——ᶜ	[66]
	10%	FPIA	COC	+	—	[68]
	10%	FPIA	PCP	+	—	[68]
Vanish®	1-10%	RIA	AMP, OPI	+	−	[66]
	1%, 10%	RIA	THC	+	−	[66]
	5%	RIA	THC	−	+++	[66]
	10%	AFIP	THC	−	+	[68]
Detergent, ionic	10%	RIA	COC	+	−	[66]
	10%, 5%	RIA	THC	+/−	+	[66]
	10%	FPIA	BAR	+/−	+	[68]
	10%	FPIA	THC	−	+	[68]
Liquid detergent	1 Drop/10 mL	EMIT®	THC	+	−/	[71]
Liquid Soapᶜ	1 Drop/5 mL	EMIT®	THC	+	−/	[71]
	10 µL/mL	EMIT®	MED	+	——	[72]
	10%	RIA	THC	+/−	+++	[66]
		RIA	PCP	+	——	[67]
	5%, 10%	RIA	THC	+	——	[67]
	5%, 10%	FPIA	PCP	−	+	[67]
	5%, 10%	FPIA	THC	−	++	[67,68]
	10%	FPIA	BAR	+/−	++++	[68]
	10%	FPIA	AMP	+/−	+++	[68]
	12 µL/mL	EMIT®	THC	+	——	[70]
	23 µL/mL	EMIT®	BAR	+	——	[70]
	42 µL/mL	EMIT®	BEN	+	——	[70]
	2%	EMIT®	THC, BEN	+/−	——	[69]
	2%	EMIT®	PCP	+	——	[69]
	2%	EMIT®	BAR	+	++	[69]
	2%	RIA	THC, BEN	+/−	++++	[69]
	2%	FPIA	AMP, BAR, THC, BEN	+/−	+++	[69]
Golden Seal	0.9%	RIA	THC	+	−−	[66]
	0.9%	FPIA	THC	+	−	[68]
	0.9%	FPIA	BAR	+/−	——	[68]
	0.9%	FPIA	AMP	−	++	[68]
	30 mg/mL	EMIT®	THC	+	——	[70]
Salt	0.25 g/mL	EMIT®	THC	+	−/	[71]
	160 mg/mL	EMIT®	MED	+	——	[72]
	50 mg/mL	EMIT®	OPI, BAR, MED	+	——	[73]
	50 mg/mL	EMIT®	AMP, COC, OPI, PCP, THC, BEN	+/−	——	[69]
	50 mg/mL	EMIT®	BAR	+	——	[69]
	75 mg/mL	EMIT®	AMP, BAR, COC	+	——	[70]
	50 mg/mL	EMIT®	OPI, THC	+	——	[70]

	10%	RIA	THC	+	—	[66]
	50 mg/mL	FPIA	BEN	+	—	[69]
	10%	FPIA	THC	+	—	[68]
Vinegar	125 µL/mL	EMIT^κ	THC	+	—	[70]
	0.5 drops/mL	EMIT^κ	THC	+	–/	[71]
	50%	RIA	THC	–	+ +	[67]
	50%	RIA	THC	+	—	[67]
	10%	FPIA	THC	+	—	[68]
Lime solvent	10%	RIA	AMP, OPI	+	—	[66]
	10%	RIA	THC	–	+ + + +	[66]
L-Ascorbic acid	10%	IRA	AMP, OPI, THC	+	–	[66]
	10%	IRA	THC	–	–	[66]
	10%	FPIA	THC	+	–	[67]
Blood	1 drop/10 mL	EMIT^κ	THC	+	–/	[71]
	10%	FPIA	THC	+/–	—	[68]
Ethanol	20%	RIA	THC	+	+	[69]
	20%	FPIA	THC	+	+	[69]
2-Propanol	20%	RIA	THC	+	+	[69]
	50%	RIA	THC	+/–	+ + +	[67]
	20%	FPIA	THC	+	+	[69]
	50%	FPIA	THC	+/–	+ +	[67]
Ethylene glycol	20%	RIA	THC	+	+	[69]
	20%	FPIA	THC	+	+	[69]
Phosphate	5%, 10%	RIA	COC	+	—	[66]
	5%, 10%	RIA	THC	+/–	+ + +	[66]
	10%	RIA	AMP, PCP	+/–	+ +	[66]
	10%	RIA	BAR	+/–	+ + +	[66]
	10%	FPIA	OPI	+	—	[68]
	10%	FPIA	PCP	+	—	[68]
Visine^u	125 µL/mL	EMIT^κ	THC	+	—	[70]
	107 µL/mL	EMIT^u	BEN	+	—	[70]
	10%	RIA	THC	+	—	[66]
	10%	FPIA	THC	+	—	[68]
Hydrogen peroxide	6 µL/mL	EMIT^u	BEN	+	—	[69]
	6 µL/mL	RIA	THC	+	+ +	[69]
	6 µL/mL	FPIA	BEN	+/–	+ +	[69]
	6 µL/mL	FPIA	THC	+	+ +	[69]

^aAbbreviations for drug categories: AMP: amphetamine, BAR: barbiturate, COC: cocaine metabolite, OPI: opiate, PCP , phencyclidine, THC: cannabinoid, BEN: benzodiazepine, MED: methadone.

^b"+" and"–" designate samples with and without the targeted analyte.

^c"+" and "–" designate enhanced and reduced response. One, two, three, and four symbols indicate slight, moderate, significant, and very significant effect, respectively. Since different measures were used for reporting interferences, the extent of interference shown in this column are gross estimates made by this author. Original articles should be consulted for more precise information.

^dNaHClO₄ is the main ingradient used in bleach preparations. Different brands were used by different investigators:

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Clorex[R] in Ref.(69-71), Cabbco in Ref.(66), and Giant Food in Ref.(67).

'The targeted analyte was believed to have been degraded(68).

'A negative result was obtained from a known positive sample. No information concerning the magnitude of the change in responses was given.

'Different brands of liquid soap were used by different investigators: Joy[R] in Ref.(69), Ivory[R] in Ref.(67), Derma Cidol 2000[R] in Ref.(66,68). The identities of the four brands used in Ref.(72) were not reported.

bleach actually caused the degradation of 9-THC-COOH[68]. Visine[R] was believed to increase the adhesion of 9-THC-COOH to the borosilicate glass specimen containers, thereby reducing the availability of 9-THC-COOH in antibody-based assays[74].

CONCLUDING REMARKS

Data provided by the reagent manufacturers and independent laboratories clearly show that results obtained from immunoassays have to be interpreted with care. Adulterants, cross-reacting compounds, and non-specific binding may generate false test results, while reagents' specificity characteristics may cause difference in quantitative results. Thus, GC/MS procedures are essential for the elimination of reporting false test results. Considering the difference in reagents' specificity characteristics, the selection of a sensible "cutoff" for an immunoassay requires careful correlation of the results obtain from the immunoassay and a GC/MS procedure.

The interpretation of positive test results also requires special knowledge and careful consideration. For example, the detection of amphetamine (or methamphetamine) in urine should not be automatically concluded as a drug abuse case-it is known[49] that licit drugs such as Amphetaminil, Clobenzorex, Ethylamphetamine, Fenethylline, Fenproporex, Mefenorex, Mesocarb, Prenylamine will generate amphetamine, while Benzphetamine, Deprenyl, Dimethylamphetamine, Famprofazone, Fencamine, Furfenorex will generate amphetamine and methamphetamine.

ACKNOWLEDGMENTS

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常用工作場所煙毒尿液篩檢之免疫學方法— 原理、交叉反應及干擾

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摘　　要

本文係探討常用市售放射免疫學方法、酵素免疫學方法、螢光偏極化免疫學方法及“粒子”免疫學方法之基本原理，此外，亦比較廠商及其他實驗室所報導之交叉反應，資料顯示，常用工作場所煙毒尿液篩檢所採用免疫學方法之特異性通常均較僅於臨床使用者高，而且，新近上市免疫學套組之特異性亦較早上市者高。最後，摻加物，如鹽、清潔劑等，對各種免疫學方法之干擾亦一併探討，惟因尚未進行更廣泛性及系統之研究，因此摻加物對於各種免疫學方法之影響及其優劣實難妄下斷語，但尿液中大麻代謝物最容易受摻加物干擾是可以肯定的。