CLIN. CHEM. 38/9, 1678–1684 (1992)

Simultaneous Detection of Seven Drugs of Abuse by the Triage™ Panel for Drugs of Abuse

Kenneth F. Buechler, S. Moi, B. Noar, D. McGrath, J. Villela, M. Clancy, A. Shenhav, A. Colleymore, Gunars Valkirs, T. Lee, J. F. Bruni, M. Walsh, R. Hoffman, F. Ahmuty, M. Nowakowski, J. Buechler, M. Mitchell, D. Boyd, N. Stiso, and R. Anderson

This novel, competitive immunoassay simultaneously detects seven drugs of abuse in urine. A urine sample is placed in contact with lyophilized reagents, the reaction mixture is allowed to come to equilibrium (10 min), and then the whole mixture is applied to a solid phase that contains various immobilized antibodies in discrete drug-class-specific zones. After a washing step, the operator visually examines each zone for the presence of a red bar. The method incorporates preset threshold concentrations that are independent for each drug. In the absence of drug or in the presence of drug in quantities less than the threshold concentration, no colored bar is visible. Samples containing drug(s) at or above the threshold concentration cause a red bar to appear for the appropriate drug(s). Positive and negative procedural control zones are incorporated into each determination. The performance of the assay methodology matches that of instrumented immunoassay systems.

Additional Keyphrases: *urine · immunoassay · colloidal gold conjugates · stereoisomers*

Classically, competitive immunoassays use ligand conjugates and antibodies in proportions that result in partial binding of the ligand conjugates in the absence of analyte (*1–5*). The measurement of the bound ligand-conjugate fraction results in a high signal in the absence of analyte, such that the signal decreases with increasing analyte concentration. The assay can be more accurate if the free ligand-conjugate fraction is measured when the free fraction is a small fraction of the total ligand conjugate, such that the observed assay signal increases with increasing analyte concentration (*5*).

Here we describe a novel immunoassay based on the use of Ascend™ Multimmunoassay™ technology (U.S. Patents 5 028 535 and 5 089 391) for simultaneous detection of multiple analytes in a sample. This technology is used in the Triage™ panel (Biosite Diagnostics, Inc., San Diego, CA) for detection of seven classes of drugs of abuse. The Triage panel contains all of the reagents necessary to perform the assay. The assay procedure takes ~10 min and is performed in three simple steps. The technology meets the growing need to provide rapid, accurate results on-site in a noninstrumented format. The method incorporates individual preset threshold concentrations for each drug. The threshold concentration for each drug is predetermined in terms of the amount of high-affinity monoclonal antibody required to completely bind the drug conjugate and the drug in the sample at concentrations up to the threshold concentration of drug. The assay response is proportional to the concentration of the unbound drug conjugate so that no signal is observed at drug concentrations less than the threshold concentration. At drug concentrations exceeding the threshold concentration, a color response is achieved. The rate of increase of the color response above the threshold concentration is a function of the relative affinities of the antibody for each of the drug metabolites and the drug conjugate and is related to the absolute affinity of the antibody for the drug conjugate. Thus, a digital response can be achieved at the threshold concentration by using antibodies having the proper affinity.

Theory

Ekins et al. (*5*) showed that the binding of a ligand or analyte by a ligand receptor or antibody selected from a group of ligand receptors may be represented by the expression

$$L + R_i = LR_i$$

where L represents the ligand or analyte and R_i represents the binding site of the *i*th ligand receptor or antibody species with $i = 1,2,3,\ldots n$. The expression describing equilibrium binding is given as

$$K_i[L][R_i] = [LR_i]$$

where K_i is the equilibrium binding constant describing the reaction in which R_i binds L. For the simplest case, in which all R_i have equal equilibrium binding constants, a closed solution for the expression can be obtained to relate the fraction of unbound ligand to the total amount of ligand for a fixed amount of receptor. This situation is of particular interest when the equilibrium binding constants, K_i, for binding of ligand to ligand receptor and for binding of ligand conjugate or drug conjugate to ligand receptor are substantially equivalent. The closed form solution for the simplest case (in which all K_i are equal) is given by Ekins et al. as

$$(F_{f/b})^2 + F_{f/b}(1 - L/R - 1/KR) - 1/KR = 0$$

where $F_{f/b}$ is the ratio of free to bound ligand, L is the total concentration of ligand, R is the total concentration of ligand-receptor binding sites, and K is the equilibrium

Biosite Diagnostics, Inc., 11030 Roselle St., San Diego, CA 92121.

Presented in part as a Poster at the 1992 Oak Ridge Conference.
Received April 17, 1992; accepted June 18, 1992.

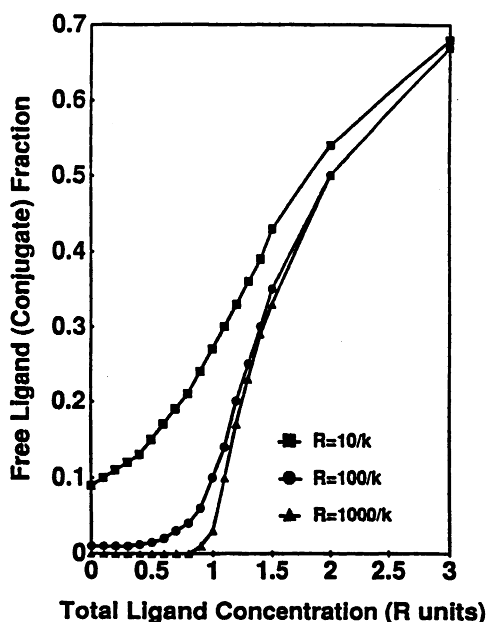


Fig. 1. Effect of the equilibrium binding constant on the assay response function

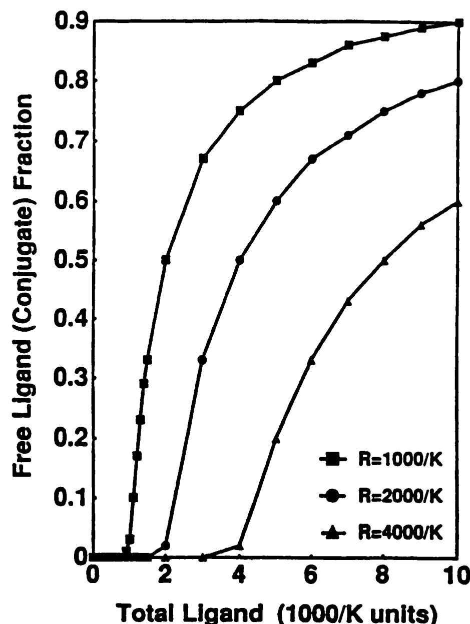


Fig. 2. Effect of the ligand-receptor concentration on the assay response function

binding constant. As the value of R increases relative to that of $1/K$ (that is, the receptor concentration is substantially greater than the dissociation constant), the functional form of a plot of free ligand as a function of total ligand concentration approaches that of a step function (Figure 1). Furthermore, the curvature at the step is related to the relationship between the equilibrium binding constant, K, and the total ligand-receptor binding site concentration, R. The free ligand fraction is directly related to the free ligand–conjugate fraction, which determines the assay response function. As R increases relative to $1/K$, Figure 1 shows that a more dramatic step increase in the free ligand or free ligand–conjugate fraction occurs. To achieve a dramatic stepwise increase in the free ligand or free ligand–conjugate fraction, one selects ligand receptors of increasing equilibrium constant, K. The relationship between the free ligand or the free ligand–conjugate fraction and the ratio of free to bound ligand, $F_{f/b}$, is given as

$$L_f/L = F_{f/b}/(F_{f/b} + 1).$$

Furthermore, when R is sufficiently larger than $1/K$, the concentration position of the step is a function of the relative value of R, the receptor binding-site concentration. As illustrated in Figure 2, increasing the value of R increases the concentration corresponding to the position of the step.

For these relationships to apply to ligand-receptor assays, the ligand conjugates and ligand receptors must be provided in such amounts that, when at equilibrium in a mixture with a sample, substantially all of the ligand conjugate is bound by ligand receptor in the absence of ligand. The amount of ligand receptor can be selected so that binding sites are present in excess of the number required to bind substantially all of the ligand conjugate. When the amount of ligand in the sample exceeds the amount of excess binding sites, then ligand and ligand conjugate compete for free receptor binding sites. The concentration of ligand in the sample that corresponds to the first detectable increase in free ligand conjugate at equilibrium is the threshold ligand concentration. As illustrated in Figure 2, the threshold concentration can be selected by appropriate choice of the concentration of ligand receptor. Thus, no response is observed until the ligand concentration exceeds its threshold. Figure 1 shows that the rate of increase of the free ligand and the free fraction of ligand conjugate as a function of total ligand concentration are determined by the equilibrium binding constant and its relationship to the threshold concentration. A suitable equilibrium binding constant is sufficient to reduce the response from the free ligand conjugate to less than the response noise of the assay.

When the equilibrium binding constants for the binding of receptor to ligand and ligand conjugate are not substantially equivalent, the slope of the response function above the threshold concentration is determined by the relative magnitudes of the respective equilibrium binding constants. When these binding constants are substantially equivalent, the response functions depicted in Figure 1 describe the assay response. When the binding constants are not substantially equivalent, the response function varies as depicted in Figure 3. When the magnitude of the equilibrium binding constant of the ligand receptor for the ligand conjugate, K^*, is greater than that for the ligand, K, the slope of the response function is decreased because more ligand is required to compete effectively with a given concentration of ligand conjugate. Conversely, when the magnitude of the equilibrium binding constant of the ligand receptor for binding to ligand conjugate is less than that for binding to ligand, the slope of the response function is correspondingly increased because less ligand is necessary to com-

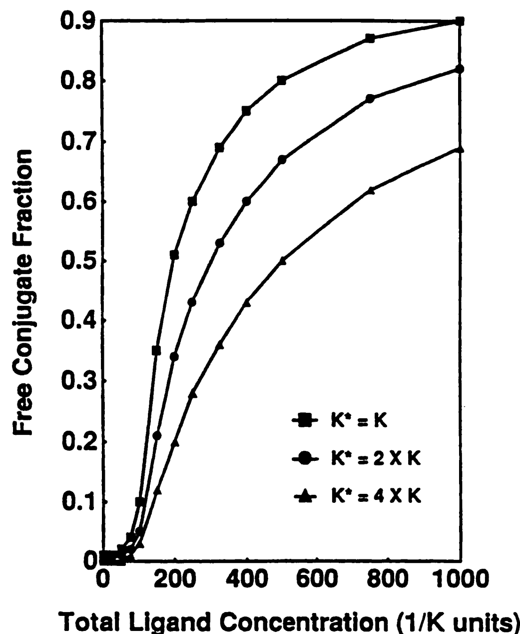


Fig. 3. Effect of the ligand-receptor-binding constant on the assay response function

pete with a given concentration of ligand conjugate.

The slope of the response function, therefore, can be varied by changing the relative magnitude of the equilibrium binding constant of the ligand receptor for ligand conjugate. This variation is most readily achieved by varying the number of ligands per ligand conjugate. It is convenient for the ligand conjugate to be labeled for detection by attaching the ligand to enzymes or colored particles. Conjugates with greater numbers of ligand per conjugate exhibit greater equilibrium binding constants for binding with the ligand receptor and have response functions with correspondingly decreased assay response slopes relative to conjugates with fewer ligands. The assay response can be empirically adjusted by this means.

Materials and Methods

Materials

The Triage panel for drugs of abuse, wash solution, and pipet were obtained from Biosite Diagnostics, Inc., as a clinical lot of product for investigational use only. Urine samples screened positive by Syva (Palo Alto, CA) EMIT® d.a.u.™ assays for the drugs of the present study at the threshold concentrations indicated were obtained from various reference laboratories in the United States. Drug-free urine samples were obtained from healthy, drug-free persons. Drug standards were purchased from Alltech Inc., Deerfield, IL, or Sigma Chemical Co., St. Louis, MO. Methoxyphenamine and β-glucuronidase were purchased from Sigma. α-Hydroxyalprazolam and α-hydroxytriazolam were gifts of Upjohn Co., Kalamazoo, MI. Temazepam glucuronide, oxazepam glucuronide, and α-hydroxyalprazolam glucuronide were synthesized enzymatically by using UDP-glucuronyltransferase (Sigma).

The reagents in the adulterant studies were obtained at a local supermarket. The UrinAid was obtained from Byrd Labs., Topanga, CA, and was used according to the manufacturer's instructions.

The reagents of the Triage panel consist of three lyophilized beads contained in the reaction well of the device. These beads contain the buffer, the antibodies, and the colloidal gold–drug conjugates. The buffer bead maintains the pH of the reaction mixture between 7.5 and 8.5. The antibody bead contains the set of monoclonal antibodies specific for the target drug metabolites in urine. The colloidal gold conjugate bead contains conjugates of drug-labeled bovine serum albumin adsorbed to colloidal gold by a modification of the procedure of Geoghegan and Ackerman (*6*). The bottom of the reaction well of the device contains a powdered mixture of citric acid and bicarbonate, separated from the three lyophilized beads by a porous plastic disc. The powder and disc create a controlled effervescence upon addition of the urine sample. The effervescence actively mixes the reagents of the reaction mixture during the incubation period.

The antibodies for the detection of the unbound colloidal gold–drug conjugates are immobilized by passive adsorption to a nylon membrane. The membrane is contained in a plastic device that is designed to maintain contact of the reaction mixture with the entire surface of the membrane before the flow of fluid through the membrane.

The wash solution contains potassium borate (100 mmol/L), sodium chloride (150 mmol/L), a detergent, and sodium azide (0.2 g/L), pH 10.

Methods

The assay protocol for the Triage panel was performed as indicated in the product insert: we added a urine sample (0.14 mL) to the reaction cup of the device, using the pipet supplied in the kit, and incubated the reaction mixture at room temperature for 10 min. Using the pipet, we qualitatively transferred the reaction mixture to the detection area and allowed the solution to drain through the membrane. We then added three drops of wash solution and let this completely drain through the membrane. The appearance of a red bar in the detection zone of a drug was read as a positive result for the respective drug; a negative result was read when no color was observed. A positive result for the Control-Positive zone and a negative result for the Control-Negative zone verified that the procedure was properly performed. The appearance of either a negative Control-Positive result or a positive Control-Negative result invalidated the determination.

For confirmation, we performed gas chromatography/mass spectrometry (GC/MS) with a Hewlett-Packard (Palo Alto, CA) Model 5890 GC and a 5970 series MS detector.[1] The instrument was operated in the splitless injection mode and the selective ion monitoring mode. We used an HP-1 (Hewlett-Packard) chromatographic

[1] Nonstandard abbreviations: GC/MS, gas chromatography/mass spectrometry; PCP, phencyclidine; and THC, tetrahydrocannabinol.

column with helium carrier gas at an average linear velocity of 50 cm/s. The urine samples containing opiates were hydrolyzed in acid (HCl, 2 mol/L, at 100 °C for 30 min), the samples containing cannabinoids were hydrolyzed in base (KOH, 1 mol/L, at 60 °C for 15 min), and the samples containing benzodiazepines were hydrolyzed with β-glucuronidase (10 kU in 0.3 mol/L potassium acetate, pH 5, at 55 °C for ≥4 h). After hydrolysis, the drug and drug metabolites were extracted in Bond Elut Certify™ columns (Varian, Harbor City, CA), according to the manufacturer's instructions. After extraction, all compounds except phencyclidine (PCP) were derivatized with the following reagents: *N*-methyl-*N*-trimethylsilyl-trifluroacetamide from Pierce Chemical Co., Rockford, IL (opiates); *N*-methyl-bis-trifluroacetamide from Pierce (amphetamine and methamphetamine); bis(trimethylsilyl)trifluroacetamide from Aldrich, Milwaukee, WI (cannabinoids, benzoylecgonine, and benzodiazepines); and Methelute™ reagent from Pierce for barbiturates, according to the manufacturer's instructions. PCP was not derivitized but was extracted as stated above.

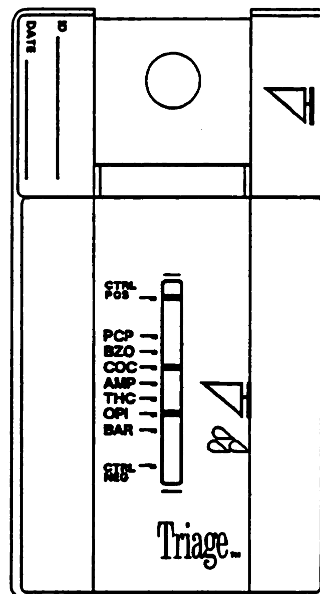


Fig. 4. Top view of the Triage device

Results

Antibody Binding Reactions

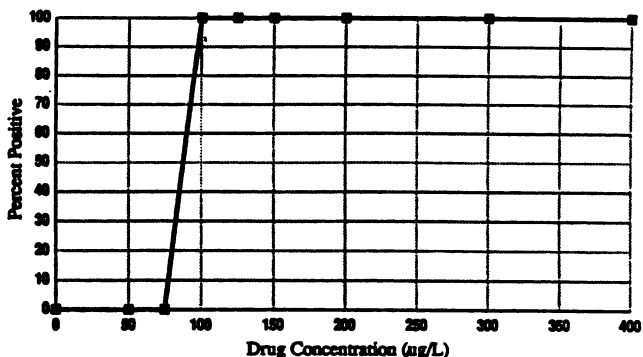
The Triage panel for drugs of abuse is a practical implementation of Ascend Multimmunoassay technology. Upon addition of the urine sample to the reaction cup within the device, the lyophilized reagents are dissolved and mixed by effervescence. A 10-min incubation allows the immunological reactions to come to equilibrium. In the absence of drug or in the presence of drug up to the threshold concentration, the quantity of antibody in the test is sufficient to bind all of the drug on the colloidal gold conjugates, resulting in bound drug conjugates. The bound drug conjugates cannot in the subsequent step bind to the antibodies immobilized on the membrane, and therefore no colored bar is observed. When drug is present in the sample at or above the threshold concentration, the drug and the drug conjugate compete for the limited number of antibody binding sites, with the result that, at equilibrium, a portion of the drug conjugate remains free. The free drug conjugates then bind to their respective antibodies immobilized in the detection zones on the membrane and one or more colored bars are then observed. Figure 4, a top view of the Triage device, represents the results of an assay that is positive for cocaine and drugs in the opiate class. The absence of a bar adjacent to the other drug classes indicates a negative result for those drugs.

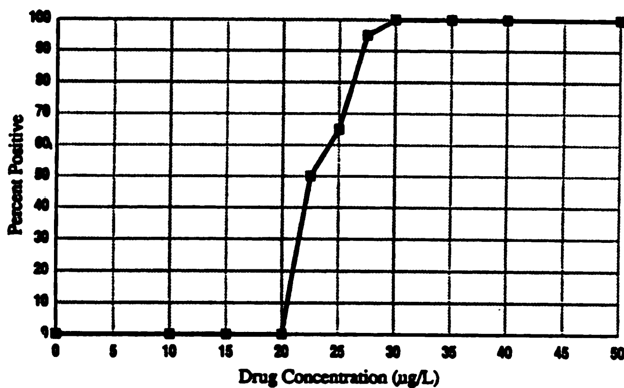
Figure 4 also shows the positions of the two procedural controls, which require specific antibody–ligand binding reactions. The device as depicted has a positive Control Positive zone and a negative Control Negative zone. For the Control Positive zone, a ligand–colloidal gold conjugate is provided that binds to the Control Positive zone upon addition of the reaction mixture to the membrane, producing a red bar. The Control Negative control includes both a ligand–colloidal gold conjugate and an antibody specific for that ligand. The amount of antibody provided is just sufficient to bind all the ligand conjugate; therefore, normally, no binding of conjugate to the Control Negative zone occurs. However, when the reaction mixture is not allowed to come to equilibrium—e.g., if the reaction mixture is added to the membrane after insufficient incubation time—free ligand conjugate will be available to bind to the Control Negative zone, resulting in the appearance of a red bar. The presence of a red bar at the Control Negative zone invalidates the assay. The assay is also invalid if no color bar is present at the Control Positive zone. Interfering substances added to the urine can interrupt the antibody/drug–conjugate binding reactions and can result in an invalid result. Procedural errors, incomplete reagents, urine adulteration, or any condition that nonspecifically alters the antibody binding reactions to the drug conjugates will invalidate the assay.

To investigate the effect of urine adulteration on the Control Positive and Control Negative zones, we adulterated urine samples with common additives and assayed them with the Triage panel. The following adulterants at the concentrations listed invalidated the assay, either by the absence of the Control Positive bar or by the presence of the Control Negative bar: Drano® at 100 g/L, Joy® at 100 mL/L, Lime-A-Way® at 100 mL/L, hydrogen peroxide at 3 mL/L, sodium chloride at 250 g/L, and UrinAid and Vanish® at 100 g/L.

Assay Performance near the Threshold Concentration

The relative affinity of the antibody for the drug conjugate and the drug dictates the slope of the dose–response curve; the threshold concentration of each drug is controlled by the antibody concentration. To test the accuracy of the Triage panel at concentrations near threshold concentrations, we performed 20 replicate assays of urine samples supplemented with selected drug concentrations. The results for tetrahydrocannabinol (THC) metabolite (*l*-9-carboxy-11-nor-Δ^9-tetrahydrocannabinol), PCP, and the cocaine metabolite ben-





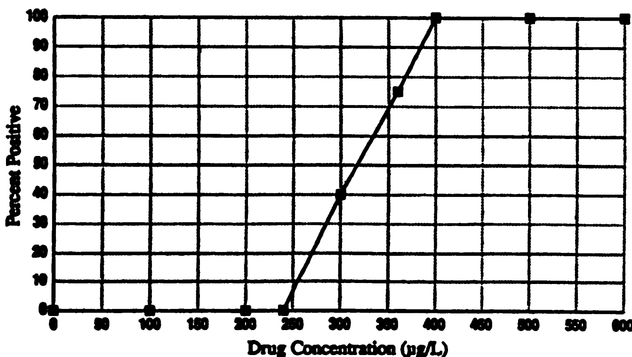


Fig. 5. Threshold analysis for the THC metabolite *l*,-9-carboxy-11-nor-Δ^9-tetrahydrocannabinol (*top*), phencyclidine (*middle*), and the cocaine metabolite benzoylecgonine (*bottom*)

Twenty replicate assays were performed of urine samples supplemented with selected drug concentrations

zoylecgonine are shown in Figure 5 (top, middle, and bottom, respectively). The slope for the results of the cocaine metabolite analysis is more shallow than for the PCP and THC metabolite analyses. This difference in slope reflects the substantially greater affinity of the cocaine metabolite antibody for the cocaine metabolite conjugate relative to its affinity for the cocaine metabolite. For assays in which lower threshold concentrations are desired, the position of the threshold curve can be shifted to detect samples containing lower drug concentrations. These results show that the onset of visible signals occurs in a narrow concentration range.

Correlation with GC/MS and EMIT Assays

The clinical specimens used for the correlation studies were judged positive by EMIT d.a.u. assays relative to the threshold concentrations used in the Triage panel for drugs of abuse. The threshold concentrations for the Triage panel are 25 μg/L for PCP, 300 μg/L for benzodiazepine metabolites, 300 μg/L for cocaine metabolite, 1000 μg/L for amphetamine, 1000 μg/L for methamphetamine, 100 μg/L for THC metabolite, 300 μg/L for opiates, and 300 μg/L for barbiturates. Table 1 compares the clinical sensitivity and specificity of the Triage panel with GC/MS and EMIT assays. The results of the three assays correlated very well.

Table 1. Clinical Sensitivity and Specificity of the Triage Panel

	No. correctly identified/total no. (%)	
Drug class	**Triage panel**	**EMIT assay**
PC		
Sensitivity	49/49 (100)	49/49 (100)
Specificity	100/100 (100)	100/100 (100)
Agreement with GC/MS	149/149 (100)	149/149 (100)
Agreement with EMIT	149/149 (100)	
Benzodiazepines		
Sensitivity	65/68 (96)	68/68 (100)
Specificity	122/125 (98)	111/125 (89)
Agreement with GC/MS	187/193 (97)	179/193 (93)
Agreement with EMIT	179/193 (93)	
Cocaine		
Sensitivity	46/47 (98)	45/46 (96)
Specificity	101/101 (100)	100/101 (99)
Agreement with GC/MS	147/148 (99)	145/148 (98)
Agreement with EMIT	144/148 (97)	
Amphetamines		
Sensitivity	48/51 (98)	48/51 (94)
Specificity	113/115 (98)	112/115 (97)
Agreement with GC/MS	161/166 (97)	160/166 (96)
Agreement with EMIT	165/166 (99)	
THC		
Sensitivity	51/51 (100)	49/51 (96)
Specificity	100/101 (99)	100/101 (99)
Agreement with GC/MS	151/152 (99)	149/152 (98)
Agreement with EMIT	150/152 (99)	
Barbiturates		
Sensitivity	49/49 (100)	45/49 (92)
Specificity	100/100 (100)	100/100 (100)
Agreement with GC/MS	149/149 (100)	145/149 (97)
Agreement with EMIT	145/149 (97)	
Opiates		
Sensitivity	46/47 (98)	47/47 (100)
Specificity	100/100 (100)	100/100 (100)
Agreement with GC/MS	146/147 (97)	147/147 (100)
Agreement with EMIT	146/147 (99)	

Antibody Specificity

The monoclonal antibodies used in the Triage panel for drugs of abuse were selected to be specific for the urinary metabolites of the drugs of abuse. This specificity for metabolites is particularly important for the benzodiazepines, opiates, and cannabinoids because these classes of drugs are metabolized and excreted primarily as glucuronide conjugates (*7–11*). Therefore, antibodies that are more specific for the parent drug may not accurately measure the substance(s) present in urine.

Table 2. Triage Panel Reactivity for Benzodiazepines, Opiates, and Cannabinoids

Drug or drug metabolite	Reactivity[a]
Benzodiazepines	
Oxazepam	800
Oxazepam glucuronide	300
Temazepam	300
Temazepam glucuronide	300
α-Hydroxyalprazolam	400
α-Hydroxyalprazolam glucuronide	300
Lorazepam	2500
Lorazepam glucuronide	400
Opiates	
Morphine	300
Morphine-3-glucuronide	500
6-Acetylmorphine	300
Codeine	300
Dihydrocodeine	300
Cannabinoids	
Δ^9-THC	1500
l-9-Carboxy-11-nor-Δ^9-THC	100
l-9-Carboxy-11-nor-Δ^9-THC glucuronide	125
Cannabinol	10 000

[a] Reactivity means that the Triage panel was judged to be visibly positive for a negative urine specimen to which the above drugs were added at the designated concentration (in μg/L) or for a clinical urine sample diluted to the appropriate concentration as determined by GC/MS. The threshold concentrations for the benzodiazepine and opiate drug classes were set at 300 μg/L, 100 μg/L for the cannabinoids. The concentrations of the glucuronide metabolites listed were determined for the parent molecules as standards and do not account for the molecular mass of the glucuronic acid.

Table 3. Triage Panel Reactivity for Barbiturates and Amphetamines

Drug	Reactivity[a]
Barbiturates	
Amobarbital	300
Butalbital	300
Cyclopentobarbital	300
Pentobarbital	300
Phenobarbital	450
Secobarbital	300
Talbutal	300
Alphenal	300
Amphetamines	
d-Amphetamine	1000
l-Amphetamine	35 000
d-Methamphetamine	1000
l-Methamphetamine	10 000

[a] Reactivity means that the Triage panel was judged positive for a drug-supplemented urine sample at the concentration listed (in μg/L). The threshold concentration for the barbiturates was set at 300 μg/L. The threshold concentrations for amphetamine and methamphetamine were set at 1000 μg/L.

Table 4. Common Cross-Reactants in Amphetamine Assays

Drug	Concn tested, mg/L	Triage panel result
l-Ephedrine	100	Negative
Phenylpropanolamine	100	Negative
Mephentermine	100	Negative
Phentermine	100	Positive
d-Pseudoephedrine	100	Negative

Table 2 shows the reactivity of the antibodies for several parent drugs and drug metabolites of the benzodiazepines, opiates, and cannabinoids. The antibody specificity for the primary metabolites is high. Assays of the glucuronides of the benzodiazepines listed in Table 2 were judged positive at the threshold concentration of 300 μg/L, whereas assays of the parent compounds were generally judged positive at concentrations higher than the threshold concentration.

The reactivity of the antibodies for the class of barbiturates is shown in Table 3. At a threshold concentration of 300 μg/L, broad specificity was demonstrated for the barbiturates listed, except for phenobarbital, which was detected at 450 μg/L. The reactivity of the antibodies to the amphetamines is also presented in Table 3. The threshold concentration was set at 1000 μg/L each for *d*-amphetamine and *d*-methamphetamine; in this detection zone, the *l*-isomers of amphetamine and methamphetamine tested negative up to 35 000 and 10 000 μg/L, respectively. Thus, the antibodies preferentially recognize the *d*-isomers of the amphetamines, which is desirable because the *l*-isomer of methamphetamine is found in some medications (*12*). Most confirmation methods currently used do not detect the different stereoisomers and thus can give false-positive results.

Several common cross-reactants in the amphetamine assays are listed in Table 4. The Triage panel produced negative results for samples containing *l*-ephedrine, phenylpropanolamine, mephentermine, and *d*-pseudoephedrine at 100 mg/L. The lowest concentration of phentermine that cross-reacted in the amphetamine assay was 100 mg/L.

These specificity results indicate that the monoclonal antibodies in the Triage panel detect the major metabolites of the drugs of abuse found in urine. The monoclonal antibodies used in the assays for classes of drugs such as barbiturates were selected for their ability to detect the entire class of compounds.

Discussion

Competitive immunoassays, including those for drugs of abuse, have been dominated by instrument-based formats. Visually interpreted competitive immunoassays have not been widely adopted because of the difficulty in developing calibration methods for such assays. Before development of the method described in this paper, visually interpreted competitive immunoassays were calibrated in two ways. The first procedure used was to develop assays with a low slope in the dose–response curve, purposefully reducing the sensitivity of the assay so that positive responses would be first observed at the threshold concentration. For example, latex agglutination assays must use this method of calibration, which results in poor discrimination of positive samples from negative samples near the threshold concentration. The second procedure for calibrating

assays used external calibrators. For example, colorimetric visual assays can also be calibrated by using separate tests of calibrators or reference color charts to compare the assay response of the sample with the response of the calibrator. Because such comparisons are subjective, assay accuracy is generally poor relative to instrument-based assays. Moreover, color comparisons become substantially more complex when assays are performed for several analytes simultaneously.

The Triage assay panel for drugs of abuse is internally calibrated through the application of Ascend Multimmunoassay technology. The ability of this assay technology to discriminate concentrations of drugs around the threshold concentration is not compromised relative to instrument-based systems. The presence of a red bar in the discrete zone specific for a drug or drug class signifies a positive result. No additional external color comparisons are necessary, and the color endpoint is stable for at least 10 min. Internal procedural controls provide additional information to the operator to ensure that each assay procedure was performed correctly and that the sample did not contain adulterants that can cause false-positive or false-negative results. The exclusive use of high-affinity monoclonal antibodies ensures reproducible sensitivity and specificity for each assay, in comparison with instrument-based systems, and lyophilized reagents provide long-term stability at room temperature. The Triage panel for drugs of abuse represents a new generation of competitive immunoassays. The Ascend Multimmunoassay technology allows for assay formats that are simple for the user and gives assay results equivalent to those for assays performed on instruments.

References

1. Yalow RS, Berson SA. Assay of plasma insulin in human subjects by immunological methods. Nature 1959;184:1648–9.
2. Yalow RS, Berson SA. Immunoassay of endogenous plasma insulin in man. J Clin Invest 1960;39:1157–75.
3. Ekins RP. The estimation of thyroxine in human plasma by an electrophoretic technique. Clin Chim Acta 1960;5:453–9.
4. Zettner A. Principles of competitive binding assays (saturation analyses). I. Equilibrium techniques. Clin Chem 1973;19: 699–705.
5. Ekins RP, Newman GB, O'Riordan JLH. Theoretical aspects of "saturation" and radioimmunoassay. In: Hayes RL, Goswitz FA, Murphy BEP, eds. Radioisotopes in medicine: in vitro studies. Oak Ridge, TN: U.S. Atomic Energy Commission, 1968:50–100.
6. Geoghegan WD, Ackerman A. Adsorption of horseradish peroxidase, ovomucoid and antiimmunoglobulin to colloidal gold for the indirect detection of concanavalin A, wheat germ agglutinin and goat antihuman immunoglobulin G on cell surfaces at the electron microscope level: a new method, theory and application. J Histochem Cytochem 1977;25:1187–200.
7. Dickson PH, Markus W, McKernan J, Nipper HC. Urinalysis of α-hydroxyalprazolam, α-hydroxytriazolam, and other benzodiazepine compounds by GC/EIMS. J Anal Toxicol 1992;16:67–71.
8. Beck O, Lafolie P, Hjemdahl P, Borg S, Odelius G, Wirbing P. Detection of benzodiazepine intake in therapeutic doses by immunoanalysis of urine: two techniques evaluated and modified for improved performance. Clin Chem 1992;38:271–5.
9. Beck O, Lafolie P, Odelius G, Boreus LO. Immunological screening of benzodiazepines in urine: improved detection of oxazepam intake. Toxicol Lett 1990;52:7–14.
10. Cone EJ, Dickerson S, Buddha DP, Mitchell JM. Forensic drug testing for opiates. IV. Analytical sensitivity, specificity, and accuracy of commercial urine opiate immunoassays. J Anal Toxicol 1992;16:72–8.
11. Williams PL, Moffat AC. Identification in human urine of 9-tetrahydrocannabinol-11-oic acid glucuronide: a tetrahydrocannabinol metabolite. J Pharm Pharmacol 1980;32:445–8.
12. Fitzgerald RL, Ramos JM, Bogema SC, Poklis A. Resolution of methamphetamine stereoisomers in urine drug testing: urinary excretion of *R*(-)-methamphetamine following use of nasal inhalers. J Anal Toxicol 1988;12:255–9.