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Essay on the Mechanics of Drug Testing

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ESSAY ON MECHANICS OF DRUG TESTING

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COLLECTION

The proper collection of the urine sample is essential for a reliable drug test. The person to be tested must provide identification, wash his hands prior to voiding, and deliver a required volume of urine within an agreed-upon temperature range. A chain-of-custody form must be initiated for each sample, and all individuals who touch the sample during the course of testing must sign this form. The person who voided the urine then releases the sample to the person responsible for collection. In the presence of the person who provided the urine sample, the collector signs the chain-of-custody form as he receives the sample and places a chain-of-custody tape over the top of the sealed and properly labeled bottle. The person who provided the sample then initials the label on the container and verifies that the sample with the identification number is actually the sample that he delivered. Using the chain-of-custody form, the collector then releases the sample bottle to a courier for transportation to the laboratory.

LABORATORY RECEIVING—ACCESSIONING

Upon receiving the sample, laboratory personnel sign the chain-of-custody form, thereby stating that they received the sample. Next, a laboratory technician inspects the sample bottle and notes any damage (such as leakage or unusual color) on the chain-of-custody form. The technician then assigns the sample an accessioning, or processing, number. A specific gravity and pH check may be performed at this time to insure the integrity of the sample. An aliquot[1] is then taken from the original bottle for screening.

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1. A portion of the specimen used for testing. 49 C.F.R. § 40.3 (1990).

SCREENING AND METHODS FOR SCREENING

The laboratory screening process varies according to the techniques used. These techniques for the detection and measurement of illegal and legal drugs of abuse in biological fluids can be divided into two categories: the immunologic method and the chromatographic method.

Immunologic techniques are relatively new tools. In contrast, thin-layer chromatography (TLC) has been in use for many years as a screening technique, but is not advisable for use in drug screening. Each methodology is unique; no single technique is all-encompassing. A good laboratory will offer and be proficient in a wide variety of testing procedures and will effectively match technology with needs.

In the discussion of drug testing methodologies, there is confusion about the terms "sensitivity" and "specificity." These words can have different meanings depending upon the person who employs them. The technical meaning of sensitivity in drug abuse testing is "how small a quantity can you detect?" The answer is usually in terms of weight (or mass) per unit volume. If one asks how sensitive a particular method is for detecting marijuana, the answer can be down to one or less than one nanogram (ng), or billionth of a gram, per milliliter (ng/ml). The term sensitivity is sometimes interchangeable with the term "detection limit." Sensitivity or detection limits vary with both the drug that is being analyzed and the analytical methodology that is being used to identify that drug.

Specificity is related to the ability of a test to exclude cross-reactivity—other drugs reacting to the test and causing inaccurate or false positive results. As the specificity of an analytical method increases, the likelihood of a false positive decreases. A combination of the proper sensitivity and specificity will provide the requisite accuracy for drug testing.

SCREENING WITH THIN-LAYER CHROMATOGRAPHY

TLC has been used traditionally as a standard "drug screen" for drugs of abuse in a number of clinical settings. It is a relatively fast and inexpensive test and does not require sophisticated instrumentation. TLC is, however, associated with an inordinate frequency of false negatives (low sensitivity) and false positives (low specificity). A major technical limitation of TLC is that the results are qualitative; that is, results are reported as either "positive" or "negative." Typically, the minimum amount of drug

or metabolite that is necessary to yield a "positive" result falls in the 1000 to 2000 ng/ml range. Thus, a negative TLC result may mean simply that the level of sensitivity of the method is insufficient to detect the drug in that sample.

In addition, TLC suffers from low specificity. The test relies on a reproducible migration pattern by the drug on a thin layer of absorbent on a glass or special paper support plate. The character and identity of an unknown drug or substance is determined by a color reaction that is produced by spraying color-changing reagents on the plate containing the unknown drug. If a drug is present in the sample, it will be identified by the distance it migrated from the bottom of the plate toward the top of the plate, as well as by the color produced in the migration. The laboratory technician subjectively detects the drug by "seeing" the substance in question with the naked eye.

SCREENING WITH IMMUNOLOGIC ASSAYS

Federal guidelines require an immunological screen.[2] The various immunologic assays, or immunoassays, operate on the principle of "antigen-antibody" interactions in biological fluids. Radioimmunoassays (RIAs), enzyme immunoassays (EIAs), and fluorescence polarization immunoassays (FPIAs) are examples of immunologic techniques and are relatively new tools used to detect drugs in biological fluids. The RIA is produced by Roche, the EIA is produced by Syva, and the FPIA is produced by Abbott. All three systems can detect the five drugs required by federal guidelines: marijuana, cocaine, opiates, amphetamines, and phencyclidine (PCP).[3] Table I illustrates the screening methods and cut-off levels.[4]

The RIA involves a gamma counter measuring the radioactivity produced by mixing urine, blood, or another biological fluid with

2. 49 C.F.R. § 40.29 (1990) (Federal Department of Transportation); Mandatory Guidelines for Federal Workplace Drug Testing Programs, 53 Fed. Reg. 11,970, 11,983 (1988) (establishing Department of Health and Human Services' scientific and technical guidelines for federal drug-testing programs other than those of the military and the criminal justice system).

3. 49 C.F.R. § 40.21; 53 Fed. Reg. 11,983.

4. A cut off level is the level at which the sample will be reported as positive for a substance. A value below the cut off level will be reported as negative. A given drug may have more than one cut off level; one level may reflect the industry consensus on what value indicates the presence of the drug, and another level may conform to the needs of a more specific purpose. See 49 C.F.R. § 40.29 for the initial cut off levels used by the Department of Transportation and 53 Fed. Reg. 11,983 for the initial cut off levels adopted by the Federal Department of Health and Human Services.

a radioactive substance. EIAs test urine directly by measuring light absorbance. The FPIA is a fluorescence technology that uses polarization units to measure drug concentration in biological fluids. Although many laboratories perform the EIA techniques, the type of instrumentation that is employed produces quantitative differences in accuracy. Sophisticated, automated instruments generally produce more accurate and reproducible results.

RIA is the oldest of the immunoassay techniques. In this procedure, the laboratory technician labels the antigen with a radioactive substance. When a urine or blood sample is combined with radioactive labeled drug (antigen) antibodies, the labeled drug and the nonlabeled drug (if present in the urine or blood sample) compete for binding sites on the antibodies. Drug presence is indicated by measuring the unbound radioactive antigen (the drug). The greater the amount of unbound labeled drug that is present, the greater the amount of drug in the sample.

An EIA contains antibodies that attach themselves to drugs or drug metabolites in a person's urine sample. The antibodies that have not attached to a drug in the sample seek out and attach themselves to a chemically tagged drug, which also is contained in the EIA test. Any leftover tagged drug produces a chemical reaction that changes the light-absorbing properties of the test mixture. The EIA test instruments measure the sample's light absorbency response, which is related to the amount of drug it contains. The more drug that is present in the person's urine, the greater the response that is produced.

FPIA is the most recent immunoassay technique adapted for the detection of drug use. FPIA involves a competitive binding reaction produced by the interaction of three components: the antibody (from the reagent system), the illegal drug (within the urine or blood sample), and the "tracer" or labeled drug (from the reagent system). The label used to tag the drug is fluorescein. A known amount of fluorescein labeled drug competes with the free drug in the sample for antibody binding sites. When bound to the antibody, the labeled drug rotates more slowly. Conversely, when the drug is free in the solution, it rotates more rapidly. The rate of rotation is determined through light intensity and is calculated into polarization units by the instrument's optics and software system. High levels of polarization correspond to low levels of the drug, and low levels of polarization correspond to high levels of the drug in the sample. The instrument measures polarization values and calculates the concentration from previously stored data.

CONFIRMATION

All chromatographic techniques can be viewed as analytical methods for the separation of complex mixtures of drugs into their individual components. Gas-liquid chromatography (GLC) and gas chromatography/mass spectrometry (GC/MS) are chromatographic techniques that are used for such confirmation. Courts have accepted GC/MS confirmation as conclusive evidence of substance use.[5] Laboratory personnel can use GLC to confirm the presence of alcohol,[6] but the technique will not withstand rigid court requirements necessary for confirming the presence of drugs.

GLC is a versatile technique that is esteemed for its sensitivity, specificity, and speed of analysis. All GLC methods consist of the following basic components: a carrier gas supply, a sample introduction inlet, a column in a temperature-controlled oven, a detector, a recorder, and the electronics necessary for control. In operation, the inlet vaporizes the sample and the carrier gas then propels the sample through the column where the mixture is separated into its components. The components pass through the detector where they are identified; the detector response is proportional to the amount of substance present in the sample.

GC/MS combines the separation versatility of a GLC detector with the specificity of the mass spectrometer and achieves a high specificity level. A mass spectrometer bombards the sample with high-energy electrons to generate extensive fragmentation ions. These ions are then plotted according to mass/charge ratio versus intensity to create a unique fragmentation pattern. Conceptually, this instrument may be considered to produce "fingerprints" of chemicals. GC/MS is the most powerful technique available; its accuracy is undisputed, and courts accept it as conclusive evidence of substance use.[7]

TESTING PROTOCOL

A technically sensible and cost effective approach requires the availability and utilization of both immunologic techniques and

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5. *See* Skinner v. Railway Labor Executives' Ass'n, 489 U.S. 602, 610 n.3 (1989) (stating that GC/MS can be a highly accurate test for the presence of drugs in biological samples).

6. *See, e.g., id.* (stating that gas chromatography can be a highly accurate test for the presence of alcohol in biological samples); Florida v. Bender, 382 So. 2d 697, 700 (Fla. 1980) (listing GLC as a method for determining alcohol intoxication and allowing executive agencies to approve breath- and blood-alcohol testing methods).

7. *See Skinner*, 489 U.S. 602.

chromatographic methodologies. A routine analysis can be automated and performed via an immunologic method. As the sample is processed for screening, the original sample bottle never leaves the secure and limited access room in which accessioning occurred. Aliquots are taken from the original sample. If an aliquot tests positive on the initial screen, it may be rescreened before being sent to confirmation. Positive immunologic results must be confirmed by GC/MS. Such an approach ensures the avoidance of false positives and yet is cost effective.

Reporting

The results of the test must be reported confidentially to protect the individual and the employer. Verbal reporting is never allowed because of a possible error. Courier service, facsimiles, and the United States Postal Service may be used if such a medium is secure and if the test results are adequately controlled at the laboratory and receiving sites.

TABLE I

DRUG	Screening Methods	Routine Screening Cut-Off (ng/ml)*	Confirmation Methods	Routine Confirmation (ng/ml)*
Cocaine	A-R-S	300[1]; 150[3]	GC/MS	150[1]; 50[3]
Marijuana	A-R-S	100[1]; 20[3]; 50[3]	GC/MS	15[1]; 20[3]; 10[3]
Opiates	A-R-S	300[1]	GC/MS	300[1]; 100[3]
Phencyclidine (PCP)	A-R-S	25[1]	GC/MS	25[1]; 10[3]
Amphetamines	A-R-S	1000[1]; 300[3]	GC/MS	500[1]; 100[3]
Methadone[2]	A-R-S	300[3]	GC/MS	300[3]; 100[3]
Barbituates[2]	R-S	300[3]	GC/MS	100[3]
Benzodiazepines[3]	S	300[3]	GC/MS	100[3]
Methaqualone[3]	R-S	300[3]	GC/MS	50[3]
Propoxyphene[3]	S	300[3]	GC/MS	100[3]
Tricyclic Antidepressants[3]	S	300[3]	HPLC	200[3]
Alcohol[3]	A-S	20[3]; 40[3] (mg/dl)**	GLC	10[3](mg/dl)**

Methods Key

A = Abbott (FPIA)
R = Roche (RIA)
S = Syva (EMIT)
HPLC = High pressure liquid chromatography

Notes Key

[1] Federal guidelines
[2] No federal guidelines for these drugs
[3] Commonly used industry standards or diagnostic standards
* unless noted, measurements are in billionths of a gram (nanograms) per milliliter: ng/ml
** milligrams per deciliter: mg/dl (dl = 100 milliliters)