Drug-use Testing: Scientific Perspectives

Kurt M. Dubowski*
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Abstract

Through a coalescence of various events and interests during the past fifteen years, a massive public concern in nonmedical drug-use has developed, resulting in increasingly widespread and large-scale drug-use testing in the United States.

KEYWORDS: drug, testing, use
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C. Relationship of drug dose, route of administration, time, and concentration
D. Limitations of drug-use testing of urine
E. Typical periods of drug detectability after use
VII. Regulation of Drug-Use Testing
A. Lack of universal accreditation or licensure systems
B. Criteria for laboratory accreditation or licensure
C. Standards for personnel of drug-use testing laboratories
VIII. Conclusions and Recommendations
A. Conclusions
B. Recommendations

I. Introduction

Through a coalescence of various events and interests during the past fifteen years, a massive public concern in nonmedical drug-use has developed, resulting in increasingly widespread and large-scale drug-use testing in the United States. Inappropriate drug use has been documented (or suspected) over the past 40 years in an increasing portion of the population, and is now considered to be a major public health and safety problem. Technological breakthroughs in drug-use testing and aggressive marketing of instruments, reagents, and materials for that purpose have widely disseminated the capability of performing simple screening tests for many categories of drugs. This has in effect removed them from the original province of a relative handful of sophisticated forensic toxicology laboratories under expert direction and control. A case can be made that this new capability, rapidly and widely implemented, has itself generated new and difficult problems for society, especially for segments such as industry, labor, and the legal system.

Drug-use testing of the U.S. military personnel was initiated on a large scale as a result of our involvement in the Vietnam war and has escalated in scale and scope, thus providing a 20 year background to its more recent expansion into the civilian sector. It is a common pattern for the rapid growth, expansion and application of new technology to actual or perceived societal problems to generate formidable new problems while solving others. The dramatic increase in the performance capabilities and availability of new clinical laboratory technology in the decade following the end of World War II is one such example. Its resemblance to many aspects of the current drug-use testing crisis is striking, especially in the problems of test demand and laboratory performance, and the issue of appropriate use of the newly available information.

Drug-use testing, often popularly called “drug testing” in the present context is, of course, only one key element in programs for control of drug use or abuse in various settings. It is useful to classify its principal elements:
1. Selection and designation of the drugs and/or drug metabolites of interest and concern;
2. Selection and collection of biofluid specimens;
3. Identification, storage, preservation, and transport of the specimens;
4. Chemical analysis of the specimens, namely, the laboratory search for the designated analytes of interest;
5. Reporting of the laboratory generated analysis results; and
6. Interpretation and use of those results.

Some cynical observers of the present drug-use testing scene would add a final seventh step - litigation (or arbitration) or other adversary proceedings, because of the growing frequency and intensity with which the results are contested. Because formal challenge of the reported test results and their involvement in litigation and other adversary processes is an expectable and predictable consequence of drug-use testing in many circumstances, the latter should be considered a forensic toxicology activity and carried out with due consideration for that status and in accordance with all of its applicable principles, procedures, and

1. The term drug-use testing, as used by the author in this article, means the systematic laboratory examination of human body fluids to determine absence or presence of drugs which are illicit or have an abuse potential, and thus to establish inferentially whether the tested person is currently using such drugs or has recently done so.
3. The American Board of Forensic Toxicology adopted the following position in November 1986: “It... is declared the policy of the American Board of Forensic Toxicology that drug (substance)-use testing activities by means of laboratory examinations be considered as encompassed within the scope of forensic toxicology when carried out under mandate of law, or under equivalent circumstances.”
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It should be recognized that not all drug-use testing is performed for the same ultimate purpose or under the same legal authority and constraints. The following applications reflect several different categories of tests:

1) Job applicants for public and private employment;
2) Employees holding federal or state civil service status or other governmental standing, including those with special status such as law enforcement authority or occupying "sensitive positions";
3) Private sector employees, including professional athletes;
4) Governmental or private sector employees or independent persons in specified occupations or specifically regulated industries, for example, railroad or aviation operating personnel and general aviation pilots;
5) Military active duty personnel;
6) Prisoners in federal civil or state custody, and parolees or probationers;
7) Individuals under lawful arrest; and
8) Incidental testees, for example, amateur athletes in NCAA-regulated programs.

Each of these categories has different characteristics with respect to voluntariness of test participation, requirements for consent, conditions of sample collection, right of access to test results, use of test results, and the consequences of positive test results or the refusal to submit to testing. The drug-use testing under discussion here and commonly performed on persons in the above categories must also be distinguished from the very different specimen collection and analysis carried out, under full evidential safeguards, as mandated or authorized by law on persons in lawful custody on criminal charges of operating a motor vehicle under the influence of or while impaired by alcohol (DUI), other drugs (DUID), or other intoxicating substances. All states and the federal jurisdiction provide for evidential testing in accordance with the statute and administrative law of the respective jurisdiction under often very specific provisions governing who may collect and analyze body fluids for those purposes and how the analyses may be conducted. It is especially important that laboratories which engage in the routine drug-use testing of job applicants and employees under consensual conditions not be misled into considering and representing themselves automatically as qualified and authorized to conduct evidential specimen procurement and analysis for investigation and prosecution of DUI and DUID offenses or other criminal charges.

Drug-use testing is currently most commonly performed on specimens of urine. That body fluid has long been routinely collected and examined clinically in connection with physical or medical examinations, whether performed for routine health assessment (as in pre-employment or periodic medical examination) or for medical diagnostic purposes. Urine collection on these occasions is, therefore, a common practice with which most persons are familiar and to which they routinely acquiesce without inquiry regarding the purpose of specimen collection. That situation lends itself to surreptitious drug-use testing on urine specimens ostensibly or perhaps legitimately collected for medical diagnostic or health assessment purposes.

In the author's view, surreptitious or clandestine testing of urine without informing the testee is wholly impermissible and unacceptable. Apart from its undeserved illegality under federal and state constitut-
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In the author's view, surreptitious or clandestine testing of urine without informing the testee is wholly impermissible and unacceptable. Apart from its undoubted illegality under federal and state constitut-
tional prohibitions against unreasonable search and seizure, it is certainly unethical conduct for any person to be knowingly administratively or professionally involved in arranging for or conducting clandestine drug-use testing. Among others, one major reason for this position is that deliberately concealed testing deprives the testee not only of guaranteed legal rights, but also of the opportunity to challenge incorrect results and any actions based thereon. One such action may be denial of employment to otherwise qualified job applicants.

Since this kind of drug-testing activity is so easily masked and concealed, there are no published data on how widespread or frequent the practice is. Clearly, it should be prohibited by specific statutory enactments; and the sooner, the better. Every person subjected to drug-use testing should be apprised of that event on every occasion, in documented fashion. No other procedure will safeguard the testee's inherent rights to challenge the test results and to refuse to undergo testing where that is a legally available option.

It is evident from the foregoing classification of testees that drug-use testing can vary greatly in the circumstances of testing and in the initial and ultimate use of the test results. The intended use of the information derived from drug-use testing ranges from the purely statistical data analysis on the nature and extent of drug presence in an anonymous population to introduction in evidence in judicial and quasi-judicial proceedings. Hence, no single or universal scheme for drug-use testing is adequate for these disparate purposes. Every drug-use testing program should, therefore, establish from the outset its purpose or purposes and the intended use of the information to be obtained. The program details and its consequent testing characteristics (such as extent, turn-around time, validity, and costs) can then be properly matched to its goals and purposes.

Among the more common applications of information obtained from drug-use testing are the following:

1) Epidemiological, anonymous data collection on drug-use;
2) Medical (including psychiatric) diagnosis and treatment of drug-dependence, addiction, or abuse;
3) Intervention, counseling, rehabilitation and other assistance to drug users;
4) Elimination of applicants for employment on the basis of recent prior illicit drug use;
5) Evidential or quasi-evidential searches on the basis of probable cause or reasonable suspicion of illicit drug use, or as a consequence of designated events (accident involvement, ex-

cessive absences from work, apparent drug intoxication or impairment) for subsequent use in administrative, disciplinary, or judicial proceedings; and

6) Validation, replication or off-setting of prior test results.

The above listed applications do not include the so-called "random testing", i.e., unscheduled, unannounced testing in the absence of clinical indications, probable cause or reasonable articulable suspicion of illicit drug use. Most so-called random testing is, in fact, not actually random. Instead, it is conditioned upon some personal or work-related characteristic of the testee such as being in a pool of social security numbers or beginning letters of last names, or in a particular work assignment or physical location. In these situations, the purpose of the test on employees is purely and simply to establish absence or presence of previously designated drugs or their metabolites with a view toward subsequent disciplinary or remedial action, termination of employment, transfer, or other administrative action. Testing every employee on the same occasion, irrespective of assignment, rank or function, would be a truly random arrangement because it eliminates opportunity for singling out given individuals (assuming all employees are present); but it is clearly impracticable. In the usual scientific sense, as in experimental design, randomization of trial, treatment or observation means that the pertinent decision is left to chance alone. In practice, true randomization is rarely obtained and that term certainly does not properly apply to the typical unannounced, unindicated mandatory drug-use testing of persons selected on the basis of personal or class characteristics.

Another troubling and unique characteristic of present private-sector drug-use testing in the United States is the complete or nearly complete absence of governmental regulation, at least with respect to its technological aspects such as selection, collection and analysis of biological specimens and interpretation of results. Nor are there universally accepted national criteria for the various elements and aspects of drug-use testing such as analysis methodology and interpretation of results. This situation is in marked contrast to the noted consensus on comparable aspects of clinical laboratory practice, where federal and state statutory and private-sector professional self-regulation are of long-standing and have had a documented positive impact on the improvement of clinical laboratory practice.

Widely prevalent attitudes reflecting this laissez-faire theory of noninterference are splendidly captured in contemporary advertisements in laboratory trade journals by a major manufacturer of drug-
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Widely prevalent attitudes reflecting this laissez-faire theory of noninterference are splendidly captured in contemporary advertisements in laboratory trade journals by a major manufacturer of drug-
use testing reagents and supplies. A large illustration depicts a thin-layer chromatogram in the process of conversion into a twenty dollar bill being extracted from a chromatography vessel; it is accompanied in the advertisement by bold headlines which proclaim "Drug screening is nobody's business but yours," and "You'll find more than hidden drugs with the..." followed by the registered trade name of the advertised drug detection system.

Unfortunately, pertinent lessons from the extensive and expensive military drug-use testing experience appear to be ignored in the civilian sector. Serious shortcomings in military drug-testing operations had been found.8 Thereafter, rigorous, universally applicable, monitored and enforced standards for the sample collection and analysis phase of drug-use testing were uniformly adopted by the military establishment. These efforts have been followed by a large decrease in contested litigation of drug-use testing results in military administrative and judicial proceedings.

II. Technology: Analytes and Specimens

Because of their mutual interdependence, analytes and specimens are paired for consideration in this article. In principle, a decision should first be made on the target drugs to be sought (i.e., the analytes) and then one or more pharmacologically and physiologically appropriate specimen materials selected for any given drug-use testing scheme. In actual practice, the decisions are often reversed. The biofluid to be collected as the specimen is often chosen on the basis of factors such as its collectability by noninvasive means or without recourse to skilled professionals. Other factors are the specimen's ability to be analyzed by a particular instrument system and the cost of the testing service. The analytes are then selected from among those which appear in the chosen specimen material, such as urine, in readily detectable form and concentration. Clearly, this is not an ideal testing situation but rather a product of compromise.


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A. Analytes

As with other aspects of drug-use testing, there is lack of unanimity on one of the most fundamental elements — what drugs should be tested for? While that sounds like a simple question, the apparent simplicity is deceptive. If the term "drug" is understood in its traditional medical sense as any substance which, when taken into a living organism, can alter or modify body functions, then the number of substances which may be considered to be drugs is vast. Some reduction in that number is achievable by excluding foods and certain other items. If one further postulates that drug-use testing in its common current context is intended to identify and ultimately to eliminate or at least limit hazards to persons, operations, property, and the public at large arising from inappropriate use of drugs by the workforce, it becomes clear that the primary targets of the testing efforts should be those drugs which have mood-altering properties as either primary or secondary characteristics. Whether this mood-altering substance is licit or illicit should not be a factor. The most prominent and ubiquitous licit drug is ethyl alcohol. It is also demonstrably the single most widely misused drug in Western societies. This wide misuse has had an enormous adverse impact upon the health, safety, and well-being of millions of persons. Yet, partly because most people merely think of alcohol as a legally available beverage and partly because of its widespread use, alcohol is generally omitted from the list of drugs to be sought in drug-use testing.

If the ultimate objective of drug-use testing is truly the promotion of health and safety by recognition and containment of drug-caused hazards, rather than simply detection of illicit-drug use as a law enforcement tool, then alcohol should clearly be included in every drug-use testing panel. This should be done despite the recognized limitation that its effect on the testee (e.g., alcohol-caused impairments) at any given time cannot be ascertained by analysis of urine. This recognized

limitation is but another indication for use of other body fluids besides urine for drug-use testing.

The selection of drugs other than alcohol for drug-use testing in different programs is variously based on one or more of the following factors: 1) prevalence of use in the population according to publicized surveys;* 2) modeling after the military drug testing programs, such as the Navy program which currently involves tests for amphetamines, barbiturates, cannabinoids, cocaine metabolite, opiates, and phenylcyclidine; 3) local assessment of target drugs based on known incidents, surveys, or other information; 4) contract proposals by drug-use testing laboratories based on their respective economic considerations or analysis capability; 5) decision to conduct on-site testing, with consequent limitations imposed by available personnel, equipment and other facilities, and analytical capabilities; 6) testing capability of a pre-selected analysis scheme, instrument, or system; 7) status as illegal or illicit substances, for example, listing in Schedules I and II of the Controlled Substances Act; and 8) testing costs. Conspicuously absent in the great majority of programs is a decision based on the previously documented frequency and severity of drug-related accidents and incidents involving death, injury or illness, property damage, product defects, or comparable misadventures.

No longer controlling the choice of drugs to be searched for are pure considerations of technology, such as lack of suitably sensitive and practical analysis methods or of drug and drug metabolite standards which until recently automatically limited the testable drug universe. National selection of target analytes should include consideration of both the potential for producing psychotropic effects and the frequency of current or recent reported use. For prescription products, popularity can be estimated from annual national prescription audits of the 200 most frequently prescribed medications.* Information is also available annually on the frequency of “Emergency Room Mentions” of both illicit and other drugs involved in overdose situations or otherwise associated with hospital emergency department patient visits, compiled by the Drug Abuse Warning Network (DAWN) operated by the Drug Enforcement Administration, U.S. Department of Justice. During


1985, the 20 controlled substances were ranked in the DAWN survey** as shown in Table 1.

Table 1. TOP 20 CONTROLLED SUBSTANCES BASED ON NATIONAL ESTIMATES OF “DAWN” EMERGENCY ROOM MENTIONS**

<table>
<thead>
<tr>
<th>Rank</th>
<th>Drug</th>
<th>Licit (L) or Illicit (I)</th>
<th>Rank</th>
<th>Drug</th>
<th>Licit (L) or Illicit (I)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Heroin</td>
<td>I</td>
<td>11</td>
<td>Lorazepam</td>
<td>L</td>
</tr>
<tr>
<td>2</td>
<td>Cocaine</td>
<td>I/L</td>
<td>12</td>
<td>Chlordiazepoxide</td>
<td>L</td>
</tr>
<tr>
<td>3</td>
<td>Diazepam</td>
<td>L</td>
<td>13</td>
<td>Phenobarbital</td>
<td>L</td>
</tr>
<tr>
<td>4</td>
<td>Marihuana</td>
<td>I</td>
<td>14</td>
<td>Oxycodone</td>
<td>L</td>
</tr>
<tr>
<td>5</td>
<td>Codeine Combinations</td>
<td>L</td>
<td>15</td>
<td>Clorazepate</td>
<td>L</td>
</tr>
<tr>
<td>6</td>
<td>Alprazolam</td>
<td>L</td>
<td>16</td>
<td>Butalbital</td>
<td>L</td>
</tr>
<tr>
<td>7</td>
<td>Amphetamines</td>
<td>I/L</td>
<td>17</td>
<td>Methadone</td>
<td>L</td>
</tr>
<tr>
<td>8</td>
<td>Phencyclidine</td>
<td>I</td>
<td>18</td>
<td>Temazepam</td>
<td>L</td>
</tr>
<tr>
<td>9</td>
<td>Propoxyphene</td>
<td>L</td>
<td>19</td>
<td>LSD</td>
<td>L</td>
</tr>
<tr>
<td>10</td>
<td>Fluazepam</td>
<td>L</td>
<td>20</td>
<td>Codeine</td>
<td>L</td>
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Another index of drug popularity is their ranking in the System to Retrieve Information From Drug Evidence (STRIDE) compiled annually by the Drug Enforcement Administration. The 1985 STRIDE listing for the same top 20 drugs found in the 1985 DAWN compilation shows that only 2.7% of all evidence exhibits consisted of licit drugs; and that the order of frequency of major controlled substances encountered as evidence was: Cocaine, marihuana, heroin, phencyclidine and PCP combinations, amphetamines, diazepam, LSD, and methaqualone.

Therefore, a case can be made, on both theoretical and practical grounds according to the present state of knowledge, that a comprehensive general drug-use testing scheme should include at least the following drugs, listed individually or as categories, and sought as parent drugs or metabolites:

* Alcohol (Ethanol)

10. See Frank, Drugs of Abuse Data, 10 ToxTalk 4 (1986).
11. Id.
12. Id.
limitation is but another indication for use of other body fluids besides urine for drug-use testing.

The selection of drugs other than alcohol for drug-use testing in different programs is variously based on one or more of the following factors: 1) prevalence of use in the population according to publicized surveys; 2) modeling after the military drug testing programs, such as the Navy program which currently involves tests for amphetamine, barbiturates, cannabinoids, cocaine metabolite, opiates, and phencyclidine; 3) local assessment of target drugs based on known incidents, surveys, or other information; 4) contract proposals by drug-use testing laboratories based on their respective economic considerations or analysis capability; 5) decision to conduct on-site testing, with consequent limitations imposed by available personnel, equipment and other facilities, and analytical capabilities; 6) testing capability of a pre-selected analysis scheme, instrument, or system; 7) status as illegal or licit substances, for example, listing in Schedules I and II of the Controlled Substances Act; and 8) testing costs. Conspicuously absent in the great majority of programs is a decision based on the previously documented frequency and severity of drug-related accidents and incidents involving death, injury or illness, property damage, product defects, or comparable misadventures.

No longer controlling the choice of drugs to be searched for are pure considerations of technology, such as lack of suitably sensitive and practical analysis methods or of drug and drug metabolite standards, which until recently automatically limited the testable drug universe. National selection of target analytes should include consideration of both the potential for producing psychoactive effects and the frequency of current or recent reported use. For prescription products, popularity can be estimated from annual national prescription audits of the 200 most frequently prescribed medications. Information is also available annually on the frequency of “Emergency Room Mentions” of both illicit and other drugs involved in overdose situations or otherwise associated with hospital emergency department patient visits, compiled by the Drug Abuse Warning Network (DAWN) operated by the Drug Enforcement Administration, U.S. Department of Justice. During 1985, the 20 controlled substances were ranked in the DAWN survey as shown in Table 1.

Table 1. TOP 20 CONTROLLED SUBSTANCES BASED ON NATIONAL ESTIMATES OF "DAWN" EMERGENCY ROOM MENTIONS

<table>
<thead>
<tr>
<th>Rank</th>
<th>Drug</th>
<th>Licit (L) or Illicit (I)</th>
<th>Rank</th>
<th>Drug</th>
<th>Licit (L) or Illicit (I)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Heroin</td>
<td>I</td>
<td>11</td>
<td>Lorazepam</td>
<td>L</td>
</tr>
<tr>
<td>2</td>
<td>Cocaine</td>
<td>I/L</td>
<td>12</td>
<td>Chlordiazepoxide</td>
<td>L</td>
</tr>
<tr>
<td>3</td>
<td>Diazepam</td>
<td>L</td>
<td>13</td>
<td>Phenobarbital</td>
<td>L</td>
</tr>
<tr>
<td>4</td>
<td>Marihuana</td>
<td>I</td>
<td>14</td>
<td>Oxycodone</td>
<td>L</td>
</tr>
<tr>
<td>5</td>
<td>Codeine Combinations</td>
<td>L</td>
<td>15</td>
<td>Clorazepate</td>
<td>L</td>
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4 Alcohol (Ethanol)


10. See Frank, Drugs of Abuse Data, 10 TOX TALK 4 (1986).

11. Id.

12. Id.
availability, accessibility and collection. Additional constraints are imposed by the nature of the information desired. A prime example of the latter is that information concerning the absence and extent of drug-produced impairment at a given time cannot be obtained solely by analysis of urine, but can be obtained from blood and its components for some drugs. Specimens potentially useful for drug-use testing are blood and its components - plasma or serum, breath, hair, saliva and urine. Other secretion such as tears, sweat, and milk (in lactating women) are minor routes for drug excretion but are not suitable specimens for mass collection.

**Blood:** Blood, in the form of whole blood, or preferably as plasma or serum, is a potentially universal specimen for drug analysis. Because the blood stream is the primary pathway for drug distribution throughout the body, presence of a drug in the blood or its components usually signifies recent intake into the body, particularly when it is found in relatively high concentration and in the form of the parent drug rather than as its metabolites or biotransformation products. For alcohol and a few other drugs, valid quantitative correlations have been experimentally established between the drug concentration in whole blood, plasma, or serum and behavioral impairments and other effects. Correlations have also been established for alcohol and a few other drugs between their concentrations in whole blood or serum and those in other body fluids and tissues, useful for some interpretive purposes. Most drugs other than alcohol are transported in blood bound to plasma proteins; hence, plasma or serum is preferable to whole blood for most drug analyses. Blood as a specimen is, therefore, best collected with sterile precautions as whole blood into a suitable container without anticoagulants or other additives; the blood is allowed to clot, and the supernatant serum removed after centrifugation to separate the cellular elements from the liquid.

Collection of blood, of course, is an invasive process. It requires penetration of the body and it is accompanied by certain risks, such as infection and prolonged bleeding in hemophilic subjects. Qualified personnel and proper supplies are necessary to collect blood. Furthermore, rather specific limitations have been imposed by the United States regulations. The specimen must be collected in a suitable container without anticoagulants or other additives; the blood is allowed to clot, and the supernatant serum removed after centrifugation to separate the cellular elements from the liquid.

13. Pharmacokinetics is the term generally applied to the study of the quantitative and temporal relationship between organism and drug, and includes consideration of absorption, distribution, localization, biotransformation and excretion of drugs.

14. Blood plasma is the liquid portion of the blood obtained by centrifugation of blood which has been prevented from clotting by addition of anticoagulants or by processing in containers with nonwettable surfaces; blood serum is the supernatant fluid obtained by centrifugation of blood after clotting has occurred.
Amphetamines: Amphetamine, Methamphetamine
Cannabinoids
Cocaine
Hallucinogens: Lysergic Acid Diethylamide (LSD), Phencyclidine (PCP), etc.
Methaqualone
Opiates: Codeine, Heroine, Hydromorphone, Morphine, Oxycodone, etc.
Prescription drugs with high potential for misuse and producing dependence: Barbiturates, Benzodiazepines, Pentazocine, etc.
Synthetic Narcotics: Fentanyl and its Derivatives, Meperidine (Pethidine), Methadone, Propoxyphene, etc.

It should be recognized that virtually any therapeutic agent (i.e., both prescription and nonprescription medications) can be misused or abused, and can produce psychic or physical dependence. That is also true for several categories of non-drug substances such as certain gases, volatile organic solvents, other inhalants, and tobacco. The variety of such non-drug materials subject to misuse and abuse has given impetus to use of the unfortunate term "substance abuse" in an attempt to be suitably inclusive.

Other classifications of analytes are extant, a common one being based on pharmacological categories such as CNS stimulants, CNS depressants, and hallucinogens. An alternative is to avoid the problem of classification altogether by simply listing the target drugs individually or by chemical drug class. Regardless of the classification scheme used to identify the target analytes of interest, they will usually consist entirely, or nearly so, of substances which exert their principal effects upon the central nervous systems, usually designated as psychoactive agents.

B. Biological Specimens

All drugs of interest in this context are absorbed into the circulation, irrespective of the route of administration, and are distributed throughout the body; thereafter they are metabolized and/or excreted. It consequently appears that one could select any accessible biofluid as the specimen for analysis. In practice, the choices are limited by the pharmacokinetics of the drug, and the practicalities of specimen availability, accessibility and collection. Additional constraints are imposed by the nature of the information desired. A prime example of the latter is that information concerning the absence and extent of drug-produced impairment at a given time cannot be obtained solely by analysis of urine, but can be obtained from blood and its components for some drugs. Specimens potentially useful for drug-use testing are blood and its components - plasma or serum, breath, hair, saliva and urine. Other secretion such as tears, sweat, and milk (in lactating women) are minor routes for drug excretion but are not suitable specimens for mass collection.

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States Supreme Court on the nonconsensual collection of blood by the States under color of law. Schmerber v. California\textsuperscript{14} holds that minor intrusions into an individual’s body under “stringently limited conditions” are permissible but such minor and more substantial intrusions under other conditions are impermissible. Clearly, it should not be assumed that Schmerber permits intrusions into an individual’s body in all settings or for other than law enforcement purposes.

**Breath:** Breath contains volatile substances such as alcohol, solvents, and their metabolites which have reached the breath by diffusion from the blood in the lungs. For very volatile substances, such as some general anesthetics, breath is the major elimination route, while for other substances it is only a minor excretion pathway. As with blood, breath concentrations of drugs reflect the instant circulating body burden and can be used to ascertain the presence or absence of impairment or other effects at the sampling time. Breath is a noninvasive specimen which is easily collectable in the field. Furthermore, it is devoid of most of the matrix problems shared by other biological specimens. The preferred breath sample is expired alveolar air. This sample is substantially in equilibrium with the pulmonary blood circulation with respect to alcohol and other volatile substances. It is most commonly and most efficiently collected as end-expired breath. This is accomplished by trapping the terminal portion of a single uninterrupted full expiration. Precautions must be taken against cooling to avoid condensation of water vapor with which the breath is saturated. This form of sampling is universally employed in quantitative evidential breath-alcohol analyzers used in traffic law enforcement.\textsuperscript{14} Since the arterial blood circulation of the lungs is essentially equal to that subsequently reaching the rest of the body and since the brain is also amply perfused by arterial blood, typically receiving about 14% of the total cardiac output, the concentration of alcohol and other volatiles in the pulmonary and brain blood supply are closely correlated. Thus, the expired alveolar breath concentration of these substances is an excellent index of their effective concentration in the brain, especially during the active absorption phase. Further, a large body of experimental data exists, correlating breath-alcohol concentrations with driver impairment, accident epidemiology and other performance data. Breath is, therefore, the specimen of preference for determination of alcohol and most other volatile sub-


stances subject to abuse.\textsuperscript{17} Certain scientific safeguards are necessary to assure the validity of breath-alcohol analysis. One safeguard is to provide a pre-sampling “deprivation” period of at least 15 minutes to eliminate the possible residual effects of ingested alcoholic beverages.\textsuperscript{18} No waiting period is required, of course, when the gas or volatile substance sought was initially inhaled rather than ingested. Any drug which has a sufficiently high vapor pressure at body temperature will reach the blood by pulmonary alveolar diffusion. However, the partial pressures or concentrations of drugs thus attained in the breath are too low to be measured by other than the most sensitive and sophisticated methods currently available (e.g., atmospheric pressure ionization mass spectrometry) or by prolonged collection of serial breaths over a sufficient time period to accumulate a detectable quantity of drug, as by absorption during breath passage through a suitable liquid solvent. Nevertheless, expired alveolar breath is a specimen of great promise which is likely to become the specimen of choice for many drugs with continued advances in analysis technology.

**Hair:** Hair has been stated to contain a historical record of past drug intake. Many drugs and other toxic substances (e.g., heavy metals like arsenic) are deposited in the hair as it grows and human hair growth generally occurs at predictable rates, typically 2.7 mm/week for head hair with a life cycle of about 3 years.\textsuperscript{18} Hair is, therefore, an accessible specimen capable of providing the longest retrospective record of drug intake. Segmental hair analysis potentially allows one to estimate when past drug intake occurred and whether past drug intake was continuous or interrupted by periods of abstinence. Naturally, the hair must be collected properly and be in sufficient quantity. Analysis of hair by such sensitive methods as radioimmunoassay following appropriate extraction has been carried out for amphetamines, cocaine and cocaine metabolite, opiates, phencyclidine, phenobarbital and other drugs.\textsuperscript{20} Although hair collection is noninvasive, the preferred method

20. See Maugh, Hair: A Diagnostic Tool to Complement Blood, Serum and Urine, 202 SCIENCE 1271 (1978); Baumgartner, Jones, Baumgartner & Black, Radioimmunoassay of Hair for Determining Opiate-Abuse Histories, 20 J. NUCLEAR MED. 748 (1979); Baumgartner, Detection of Phencyclidine in Hair, 26 J. FORENSIC
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With sensitive modern analytical methods, drugs can be identified and quantitated in as little as 20-50 microliters of saliva, which are readily and rapidly collectible without prior preparation. The additional advantages - identifying the specimen donor with certainty, eliminating contamination, dilution or other specimen alterations or tampering during the collection process, and obviating unacceptable invasion of privacy during specimen collection make saliva a potentially very practical specimen for drug-use testing schemes. Some additional research is necessary to extend our knowledge of what drugs of interest appear in the saliva. For example, we must discover whether there are secretor/nonsecretor differences for some drugs, the relative concentrations of conjugated or bound versus free drugs in the saliva, and other relevant data.

It is easy to visualize extension of saliva analysis for drugs to large-scale studies on the pharmacokinetics and the time-related effects of drugs, which are necessary to understanding drug-induced impairments. An obvious extension of analytical technology to saliva analysis is to develop solid-phase tests for key drugs, in a form analogous to the "tipsticks" widely used in urine testing. Commercial versions of these solid-phase tests have been developed for alcohol in saliva and are now on the market. They use enzymatic oxidation with alcohol oxidase and an indicator dye whose blue color intensity semi-quantitatively reflects the salvia-alcohol concentration. A substantial amount of experimental work has been carried out on saliva alcohol analysis since the 1930's, and high correlation of whole blood, plasma and serum alcohol concentrations with those in saliva has been demonstrated.

Saliva has thus been shown to be an appropriate specimen for determination of alcohol for experimental studies, clinical applications and very probably for certain forensic uses. Presence of cannabinoids in mixed saliva has been demonstrated, by radioimmunoassay for Δ⁹-THC and by other methods, after both recent smoking and ingestion of marijuana. Cocaine is present in mixed saliva after intravenous adminis.
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tration as well as after oral intake. There are also other abused drugs which have been demonstrated in saliva. They include the following: amphetamine; anticonvulsants; barbiturates; benzodiazepines including diazepam and its major metabolite; LSD; methaqualone; opiates including codeine, heroin, and morphine; steroids; major tranquilizers; and several volatile substances in addition to ethanol. The potential applicability of drug detection in saliva to the evaluation of impaired drivers has also been demonstrated in a pilot study in which benzodiazepines, cannabinoids, and cocaine were detected in mixed saliva, in addition to caffeine and nicotine. It is apparent that saliva is a very versatile specimen which has not yet been used optimally and which seems to be far more than an ultrafilter of plasma.

Urines. Urine has become the de facto principal specimen material employed in drug-use testing by the military establishment and the civilian sector despite material shortcomings. This present preeminent position was reached chiefly for pragmatic reasons and because of the circumstance that the early commercial developments in drug testing instrumentation and reagents capable of large scale application (e.g., the Abuscreen System of Roche Diagnostic Systems, and the FRAT and EMIT Drug Detection Systems of Syva Company) were limited to analysis of urine specimens. Urine had been the most commonly used biological sample in the forerunner of multiphasic drug-use testing; it was originally used in the late 1960s and early 1970s for the diagnosis of heroin addiction and day-to-day management of patients enrolled in methadone maintenance programs.

As indicated earlier, whatever the entry route of a drug, it is carried by the blood stream to the brain, liver, kidney and other organs and the process of metabolism and excretion begins. Metabolism or bioconversion of most drugs changes them, at least partly, to conjugated forms which are more water soluble than the parent drug and hence excreted primarily through the kidneys into the urine. That process is controlled by various pharmacological, biochemical, and physiological factors, including the chemical nature of the metabolites and conjugates as well as the pH of the urine and other factors including the drug concentration in plasma. Because bladder urine is a pooled specimen accumulated over time, and because the kidneys in effect concentrate the urine with respect to many constituents including most drugs, the concentration of most drugs excreted in urine is higher than in other accessible biological fluids at the same time. These facts, and the noninvasive nature of urine collection by micturition, the relatively large volumes of urine available by ordinary voiding compared with practically collectible volumes of blood or saliva, and the usually high stability of drugs in urine, have combined to make urine the most common specimen for drug-use testing.

There are also substantial disadvantages to urine as a drug specimen. Direct visual observation of urine collection has been amply proven to be necessary to connect the urine specimen to a given testee. Direct visual observation is needed to prevent substitution of other specimens for the authentic urine and to eliminate post-voiding tampering by the testee with the urine by dilution or addition of substances which will interfere with the analysis. Such direct monitoring of the act of urinary voiding is unpleasant for the observer and observed alike. Furthermore, it often impedes voluntary voiding in many persons.

Dilution of urine to reduce drug concentrations below readily detectible concentrations can be accomplished not only by the addition of water, drug-free urine, or other diluents to an authentic voided urine specimen. It can also result from copious fluid intake prior to urination. Some drugs are not excreted in urine as the parent compounds introduced into the body but only as metabolites which may not specifically indicate which one of the given class of drugs (e.g., opiates) was administered. The urine drug concentration often varies greatly among individuals receiving the same drug dose. Urine drug concentrations can also vary greatly in single urine specimens from the same person on different occasions, depending on factors such as fluid intake, state of hydration, urinary pH and the functional circulating plasma concentration of the drug concerned. The latter is a function of drug dose, elapsed time since administration of that drug dose, and individual variations in drug metabolism. The drug quantity administered by whatever route, its bioavailability (i.e., capacity to be absorbed into the bloodstream from the administered state), and the elapsed time since...
tation as well as after oral intake. There are also other abused drugs which have been demonstrated in saliva. They include the following: amphetamine; anticonvulsants; barbiturates; benzodiazepines including diazepam and its major metabolite; LSD; methaqualone; opiates including codeine, heroin, and morphine; steroids; major tranquilizers; and several volatile substances in addition to ethanol. The potential applicability of drug detection in saliva to the evaluation of impaired drivers has also been demonstrated in a pilot study in which benzodiazepines, cannabinoids, and cocaine were detected in mixed saliva, in addition to caffeine and nicotine. It is apparent that saliva is a very versatile specimen which has not yet been used optimally and which seems to be far more than an ultraliter of plasma.

Urine. Urine has become the de facto principal specimen material employed in drug-use testing by the military establishment and the civilian sector despite material shortcomings. This present preeminent position was reached chiefly for pragmatic reasons and because of the circumstance that the early commercial developments in drug testing instrumentation and reagents capable of large scale application (e.g., the Abuscreen System of Roche Diagnostic Systems, and the FRAD and EMIT Drug Detection Systems of Syva Company) were limited to analysis of urine specimens. Urine had been the most commonly used biological sample in the forerunner of multiphasic drug-use testing; it was originally used in the late 1960s and early 1970s for the diagnosis of heroin addiction and day-to-day management of patients enrolled in methadone maintenance programs.

As indicated earlier, whatever the entry route of a drug, it is carried by the blood stream to the brain, liver, kidney and other organs and the process of metabolism and excretion begins. Metabolism or bioconversion of most drugs changes them, at least partly, to conjugated forms which are more water soluble than the parent drug and hence excreted primarily through the kidneys into the urine. That process is controlled by various pharmacological, biochemical, and physiological factors, including the chemical nature of the metabolites and conjugates as well as the pH of the urine and other factors including the drug concentration in plasma. Because bladder urine is a pooled specimen accumulated over time, and because the kidneys in effect concentrate the urine with respect to many constituents including most drugs, the concentration of most drugs excreted in urine is higher than in other accessible biological fluids at the same time. These facts, and the noninvasive nature of urine collection by micturition, the relatively large volumes of urine available by ordinary voiding compared with practically collectible volumes of blood or saliva, and the usually high stability of drugs in urine, have combined to make urine the most common specimen for drug-use testing.

There are also substantial disadvantages to urine as a drug specimen. Direct visual observation of urine collection has been amply proven to be necessary to connect the urine specimen to a given testee. Direct visual observation is needed to prevent substitution of other specimens for the authentic urine and to eliminate post-voiding tampering by the testee with the urine by dilution or addition of substances which will interfere with the analysis. Such direct monitoring of the act of urinary voiding is unpleasant for the observer and observed alike. Furthermore, it often impedes voluntary voiding in many persons.

Dilution of urine to reduce drug concentrations below readily detectable concentrations can be accomplished not only by the addition of water, drug-free urine, or other diluents to an authentic voided urine specimen. It can also result from copious fluid intake prior to urination. Some drugs are not excreted in urine as the parent compounds introduced into the body but only as metabolites which may not specifically indicate which one of the given class of drugs (e.g., opiates) was administered. The urine drug concentration often varies greatly among individuals receiving the same drug dose. Urine drug concentrations can also vary greatly in single urine specimens from the same person on different occasions, depending on factors such as fluid intake, state of hydration, urinary pH and the functional circulating plasma concentration of the drug concerned. The latter is a function of drug dose, elapsed time since administration of that drug dose, and individual variations in drug metabolism. The drug quantity administered by whatever route, its bioavailability (i.e., capacity to be absorbed into the bloodstream from the administered state), and the elapsed time since
the drug was taken are the principal factors affecting the drug concentration in the plasma. It and the pooled urine volume are the principle factors which influence the drug concentration in voided specimens of bladder urine. None of the foregoing factors are under control of the collector. With some drugs, body stores accumulate and are gradually released even after cessation of intake that drug, so that positive urine test results are obtainable days or even weeks after last drug administration. Cannabis (marihuana) is the leading example.

The principal features of urine as a specimen for drug analysis are summarized in Table 2.

Table 2. Urine as a Specimen for Drug Tests

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<td>Readily substituted, diluted or otherwise altered, unless collected under direct observation</td>
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<td>Available in large volumes</td>
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<td>Urinary drug and metabolites are stable</td>
<td>Lack of correlation of urine drug concentrations with impairment or other effects at time of collection or other relevant time</td>
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<td>Readily preserved by freezing or chemical preservatives</td>
<td>Absence of parent form of many drugs impedes differentiation among alternate administered drugs</td>
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<td>Cellular material and proteins not commonly present in great quantities, simplifying the analysis</td>
<td>Difficulty in voluntary urine voiding in many persons upon command, especially under observation</td>
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<td>Wide availability of commercial reagents and systems for drug detection in urine</td>
<td>Privacy invasion when urination is observed</td>
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<td>Elevated drug concentrations compared to other biological materials</td>
<td>Urine is subject to decomposition if not promptly and properly treated and refrigerated/autoclaved</td>
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<td>Presence of metabolites can indicate drug use by subject</td>
<td>Urine drug concentrations are not well correlated with those in other body fluids</td>
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<td>Large literature base on urine drug analysis and significance of analysis results</td>
<td>Analysis may be deceptively &quot;simple,&quot; leading to errors</td>
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Quantitation of analytes in urine is an essential step in the analysis procedure to determine whether the established concentration threshold for positive results has been reached or exceeded; it can be performed directly or indirectly. For consistency in interpretation of results, both among different individuals and in the same person on different occasions, it is desirable and good laboratory practice to minimize the effect of fluctuations in the urine water content reflecting urinary flow rate and the other factors mentioned above. That can be accomplished readily by relating the drug quantity to a unit quantity of urinary creatinine rather than to a unit volume of urine. Creatinine is a product of endogenous metabolism which is neither actively secreted nor absorbed by the kidneys. Therefore, it can be used as a "marker" to adjust for urine flow fluctuations, that is, to "normalize" drug concentrations by expressing them in units of, say, nanograms per milligram of creatinine rather than ng/ml of urine. Even so, results of drug tests on urine can only indicate absence or presence and concentration of target analytes. Those results cannot be used, by themselves, to establish exactly when or what dose of a drug was administered. Neither can they be used to draw valid inferences regarding the systemic concentration of a drug at the time of urine collection or any prior time, or about the absence or presence and extent of drug produced impairment or other systemic effects. This is a marked contrast to the significance of plasma or saliva drug concentrations.

It is an indication of the ubiquity of urine drug testing that two monographs have been issued on "Urine Testing for Drugs of Abuse" by the Federal government, one in 1973 and one in 1986. Other Specimens: Specimens other than the biological samples discussed above can be analyzed for drugs but are not involved in customary drug-use testing. Since drugs are carried in blood, urine, saliva, semen, sweat, tears, and other biofluids, stains produced by any of these fluids on cloth or other absorbent materials can in principle be analyzed for drugs by sufficiently sensitive methods. Such testing is

31. See NATIONAL INSTITUTE ON DRUG ABUSE, DEPARTMENT OF HEALTH AND HUMAN SERVICES, PUB. NO. (ADM) 87-1481, URINE TESTING FOR DRUGS OF ABUSE (1986).
32. See Smith & Pompozini, Detection of Phenobarbital in Bloodstains, Semen.
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III. Technology: Drug Analysis Methods

Space considerations preclude other than a cursory discussion of the principles of analytical methodology for abused drugs, and method characteristics.

No single analysis method or technique is capable of encompassing a search for all analytes of interest in drug-use testing - drugs and drug metabolites - and of measuring the pertinent physiological, physical or chemical characteristics of interest or concern in every drug testing scheme, program, or circumstance. Neither can all analysis methods be universally applied to all biological specimen materials. Some methods are only applicable to a given biological specimen, for example, urine, in their original version. Others are more versatile and can be used without extensive modifications for various specimen materials. Still others can be suitably modified for use with a given biological material.

A. Analysis Methods

Test Category: The analysis aspect of drug-use testing in the context of this article can be considered as a two-stage process - "screening" or presumptive testing, followed by confirmatory analysis on positive specimens. Screening or presumptive tests are, in general, initial sorting procedures to eliminate from further consideration those specimens which are drug free or contain drugs only below established thresholds, and secondarily to indicate for further consideration those specimens which apparently contain one or more identified target analytes at or above their respective threshold concentrations. A threshold concentration is sometimes referred to as "cutoff." In practice, an instrument response or other analytical method output above the inherent sensitivity limit of the method but below the calibrated "cutoff" concentration is interpreted as a negative result. Response or output equal to or greater than the "cutoff" is considered to be a positive result.

Positive presumptive results of screening tests, standing alone, lack the validity necessary for use in strictly forensic applications, such as driving under influence of drugs prosecutions and for use in other proceedings which will or can adversely affect the testee, such as disciplinary action by employers, denial of employment to job applicants, or for most other applications other than anonymous studies of drug use. Accordingly, all results of these types of screening tests must be properly verified (or superseded) by subsequent confirmatory analyses. The only time this isn't necessary is when the methods used for the initial screening tests were themselves of confirmatory test quality.

In general, the confirmatory analysis must be more reliable than the presumptive or screening test. In particular, if the confirmatory test result is to be valid and meaningful, it must either be specific or more selective for the target analyte and must be at least as sensitive as the initial screening test. The current informed professional consensus on the requirements for confirmatory testing is reflected in the following position statement adopted by the Toxicology Section of the American Academy of Forensic Sciences in 1986:

Confirmation of results is essential in forensic toxicology. Positive results of toxicological screening tests, regardless of the method used, and positive toxicological analysis results obtained by immunoassay methods should either be adequately confirmed before the results are used for forensic purposes, or be clearly designated as "unconfirmed" results.

Analysis methods used for attempted confirmation of presumptive results must be appropriately sensitive and specific or unequivocally selective for the analyte(s) in question, and must be based upon different chemical or physical principles than the initial analysis method(s).

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34. See McBry, Dubowski & Finkle, Urine Testing for Marijuana Use, 249 J.A.M.A. 881 (1983); Catlin supra note 30; National Institute on Drug Abuse, Department of Health and Human Services, supra note 31.

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B. Characteristics of Analysis Methods

All analytical methods can be characterized in terms of two groups of characteristics - reliability and practicability. The former concerns the appropriateness, validity and correctness of results yielded by the method, while the latter concerns applicability, usability and utility of the method in a given situation. Table 3 lists the major factors relating to both characteristics.

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Accuracy: Accuracy means concordance between an experimental result and the true fact or value. The term applies to both qualitative and quantitative results. Clearly, accuracy — the correctness of the result reflecting the true situation — is the supreme requirement for an analysis. A qualitative test must correctly indicate either absence of all target analytes or their presence and identity. An incorrect qualitative result can be either a false positive, indicating apparent presence of a drug or drug category which is not in fact present; or a false negative, indicating apparent absence of a drug or drug class which is in fact present in the specimen at or above the pre-established detection limits or "cutoff" values. Incorrect quantitative results misrepresent the concentration of a particular analyte in the specimen. Errors can either be systematic or random. The former is a predictable and observable characteristic of a method which regularly yields biased results. The latter is usually an accidental and unpredictable aberration which can be caused by failure to adhere to the analysis protocol, computation errors, or other inadvertence.

Systematic errors can be revealed by including appropriate use of control specimens of known composition in every set of analyses, or by result comparisons on proficiency test specimens and by other quality control measures. Random errors can generally only be detected by repeated careful analysis of the same specimen carried out from the start. Hence, it is good laboratory practice automatically to repeat analyses of a fixed proportion (e.g., 5 per cent) of all specimens of sufficient quantity, with comparison of the independently obtained results. Lack of agreement in these results is an indication that the analysis system is prone to random error. With quantitative methods, accuracy signifies the closeness of a measured value to the true value. It can be assessed by the assay of specimens with independently established reference compositions, or by comparison of a laboratory's result with the mean.
value of results on identical proficiency test (P.T.) specimens, compiled from a sufficiently large cohort of participant laboratories or reference laboratories in a P.T. survey program, and by other measures. The term “uncertainty” denotes an estimate by statistical means of the bounds of inaccuracy; it reflects the fact that few experimental measurements will coincide absolutely and completely with the true value each and every time; and it affords a statistical measure of the limits between which a true value is likely to lie.

All chemical, physical and biological analyses are subject to error. They can differ in the nature of errors which can occur, the magnitude and extent of possible errors, and the probability of the occurrence of both systematic and random errors.

**Precision:** Precision is the reproducibility of results from quantitative measurements; it refers to the variability of the individual results of replicate (repeated) measurements of the same specimen. A method which shows small scatter is considered to be precise, while wide scatter signifies imprecision. Observed scatter of measured values is usually the result of random errors, that is, each individual value will tend to deviate from the true value. Since the probability of occurrence of a zero random error is zero, it is unlikely that any individual measurement made by an unbiased system will be completely accurate.

Replicate measurements add confidence to the mean result and increase its likely accuracy. An analysis method should be sufficiently precise to minimize the number of repeated measurements required for the intended use. The greater the inherent precision, the fewer replicate analyses are needed to provide data which would not be significantly improved by further replication. Full disclosure of the analytical method characteristics used by a drug testing laboratory should include statistical measures of precision such as the Coefficient of Variation for replicate analyses at a stated mean concentration, determined in the laboratory itself, not simply obtained from the literature, a manufacturer, or other secondary source.

**Specificity:** Specificity is a most important characteristic of drug analysis methods. In chemical terms, specificity is response by a method only to the particular target substance sought; consequently it is an absolute term - a method is either specific or it is not specific for a given analyte. In practice, the “specificity” of a test can be considered as its ability to distinguish unequivocally between the compound or compound class (e.g., barbiturates or opiates) to be measured and other, closely related drugs, metabolites, or naturally occurring substances which are present in the specimen matrix. For some purposes, as in initial screening tests, “specificity” limited to a drug class (e.g., barbiturates, or amphetamines, or opiates) rather than to an individual drug is an advantage. For example, cross-reactivity of an immunochemical assay with both morphine and its morphine glucuronide metabolite can enhance the sensitivity of the assay for that target drug without need for pre-test preparation such as hydrolysis of the specimen; or cross-reactivity to codeine, morphine, hydromorphone and other opiates can indicate by a single test whether any drug in that class is present. Inappropriate nonspecificity, however, leads to false positive results.

**Selectivity** can be considered as less than absolute specificity, that is, the ability of a method “selectively” to respond more readily to a desired specimen constituent or target analyte than to other constituents present in the specimen, whether target analytes or not. This selectivity can be manifested by response to a lower concentration of the target analyte (drug or drug class) than of other drugs or non-drug physiological specimen constituents, or ability to respond more rapidly or more completely so as to discriminate among various target drugs. Cross-reactivity of an immunochemical assay to all drugs and their metabolites in a given class (e.g., opiates) exemplifies selectivity rather than specificity. Specificity implies the ability to discriminate, for example, between codeine, morphine, hydromorphone and other opiates. Inability of a method to distinguish between different classes of drugs (say, opiates versus synthetic narcotics) constitutes nonspecificity.

A phenomenon related to specificity and selectivity is **interference.** Interference signifies that a constituent of the specimen other than the target analyte under investigation can or did cause response in an analysis. In true interference, it is generally unrecognized that a result was caused or was affected by the substance(s) other than the analyte to which the response is ascribed. While the usual outcomes of interference are a false positive qualitative result or a falsely elevated quantitative result, negative interference can also occur. Such interference prevents response by a target analyte which is actually present or diminishes the true quantitative result. Some immunochemical assays, for example, are subject to negative interference by addition of sodium chloride or other substances to a urine specimen, usually in tests which do not involve preparatory steps such as extraction or other separational procedures. Interferants or interfering substances are those constituents of a specimen which cause responses similar or identical to that of the target analyte or otherwise alter the results. So-called “matrices effects,” responses caused by physiological specimen constituents...
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Selectivity can be considered as less than absolute specificity, that is, the ability of a method "selectively" to respond more readily to a desired specimen constituent or target analyte than to other constituents present in the specimen, whether target analytes or not. This selectivity can be manifested by response to a lower concentration of the target analyte (drug or drug class) than of other drugs or non-drug physiological specimen constituents, or ability to respond more rapidly or more completely so as to discriminate among various target drugs. Cross-reactivity of an immunochromatographic assay to all drugs and their metabolites in a given class (e.g., opiates) exemplifies selectivity rather than specificity. Specificity implies the ability to discriminate, for example, between codeine, morphine, hydromorphone and other opiates. Inability of a method to distinguish between different classes of drugs (say, opiates versus synthetic narcotics) constitutes nonspecificity.

A phenomenon related to specificity and selectivity is interference. Interference signifies that a constituent of the specimen other than the target analyte under investigation can or did cause response in an analysis. In true interference, it is generally unrecognized that a result was caused or was affected by the substance(s) other than the analyte to which the response is ascribed. While the usual outcomes of interference are a false positive qualitative result or a falsely elevated quantitative result, negative interference can also occur. Such interference prevents response by a target analyte which is actually present or diminishes the true quantitative result. Some immunochromatographic assays, for example, are subject to negative interference by addition of sodium chloride or other substances to a urine specimen, usually in tests which do not involve preparatory steps such as extraction or other separational procedures. Interferers or interfering substances are those constituents of a specimen which cause responses similar or identical to that of the target analyte or otherwise alter the results. So-called "matrix effects," responses caused by physiological specimen constituents
(e.g., proteins or electrolytes), rather than by the target analyte, likewise constitute a form of interference in the analysis, whether they enhance, suppress or otherwise alter the results.

Sensitivity: In drug-use testing, sensitivity is commonly defined as the minimal concentration of an analyte in an undiluted biological specimen which is detectable with high probability. For other scientific purposes, sensitivity is often stated in terms of the absolute quantity of analyte detectable with high probability or certainty by a given method, sometimes in the form of the pure analyte. Obviously, the required sensitivity depends upon the purpose of the analysis. It has been suggested that sensitivity in drug-use testing be defined as the concentration of a particular analyte detectible by a given method in a specified specimen material 99% of the time, as a value readily determined statistically with given confidence limits by simple experiments.

A complication arises in characterizing the sensitivity of analysis methods which give continuous quantitative results, such as some immunochemical assays. Minimum "cutoff" concentrations or instrument responses are commonly selected to reflect the operational sensitivity of these methods for use as screening tests. Assay values at and above the "cutoff" are considered to be positive results, while those below the "cutoff" are deemed to be negative results. These thresholds must be selected with appropriate consideration of the upper limit of assay values yielded by the relevant normal and abnormal drug-free biological specimen from a sufficiently large group of individuals of both sexes, various ages, and other relevant demographic characteristics. It is also necessary that attention be given to "nonspecific" assay responses caused by other factors than the target analyte. At any specified concentration, the scatter or distribution of values depends on the precision or variability of the assay; hence the concentration of a target drug detectible at a rate of 95% (or higher) will be greater than the "cutoff" value, assuming Gaussian distribution of the scatter.

It should be noted in passing that the terms "sensitivity" and "specificity" have different meanings when applied as statistical indices of the efficiency of a diagnostic test used in clinical medicine.


Applications of that type, sensitivity and specificity are defined as follows, expressed as a numerical ratio (or converted into a percentage):

\[
\begin{align*}
\text{Sensitivity} &= \frac{\text{Number of True Positive Results}}{\text{Number of True Positive Results} + \text{Number of False Negative Results}} \\
\text{Specificity} &= \frac{\text{Number of True Negative Results}}{\text{Number of False Positive Results} + \text{Number of True Negative Results}}
\end{align*}
\]

Detection Limit: This is often defined as the smallest quantity (or concentration) of an analyte which can be reliably detected, by a given method, in a single analysis and with a sufficiently high level of confidence to be independent of the statistical fluctuations to which every method is subject. The usual form of that definition is the analyte quantity (or concentration) which provides a ratio of at least 2 to 1 for method response to the target analyte/nonspecific method response of the same specimen lacking the target analyte. In effect, this detection limit involves the concept of blank value or blank response, the measured value or method response yielded by a given biological specimen material which is unequivocally free of the target analyte. Such "blanks" must be routinely analyzed as part of each set of analyses (or together with any single analysis). This provides assurance that no false positive results will be produced and provides a "blank" result value to be subtracted from each target analyte response before the net measured value, attributable to the target analyte is used to determine the final result. In drug-use testing, absolute detection limits for pure analytes in nonbiological matrices are generally meaningless and the relevant value for every method in use should be determined for each biological specimen material to be tested. Although not truly applicable in analytical chemistry, the electronics term "signal to noise ratio" is often used to denote the analogous chemical situation of target analyte response over nonspecific background (or "blank") response. Obviously, the concepts of sensitivity and detection limit are related.

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**Resolution:** This term denotes the ability of a method to discriminate unequivocally between closely adjacent concentrations of the target analyte.
get analyte in a biological specimen. It is, therefore, related to the sensitivity of the method and to its precision. Adequate resolution is required for quantitative measurements in which a specified threshold value determines the significance of a result and the gravity of its consequences. That is true, for example, for statutorily established blood-alcohol concentrations as the alcohol element of per se (or absolute concentrations) driving-under-the-influence laws. Resolution in quantitative measurements is thus somewhat analogous to the specificity characteristics of qualitative analysis methods.

**Practicability Parameters:** The method characteristics listed in Table 3 under "Parameters Affecting Practicability" are largely self-defining and self-explanatory. There is commonality or interdependence between some of these parameters. Costs, for instance, are clearly determined in part by the nature and extent of personnel involvement in a given kind of analysis as well as the level of education, training, and experience required of the analyst. None of these characteristics can be evaluated in isolation.

The turn-around time is one example. It refers to the length of time required to complete the analysis of one sample, assuming all necessary facilities and supplies are at hand. However, drug-use testing is not usually carried out on one sample at a time. Instead, drug-use testing is generally done in groups or sets or in an essentially continuous sample chain fashion. The individual turn-around time is thus not nearly as significant as the total number of specimens which can be processed in a given time period; such as an 8-hour work shift. Some testing methods require a 24-hour interruption for one step in the analysis. Other testing methods may be so lengthy that an analysis cannot be completed in one normal work shift, thereby requiring involvement of different analysts with any given test. Stability factors for reagents and calibration also affect how rapidly a given sample can be tested on an intermittent test performance basis. Ultimately, this can affect the timely availability of results as well as overall costs.

Those parameters listed and other practicability considerations need to be evaluated for a given laboratory environment. Unfortunately, few published methods include the relevant practicability parameter information in the literature. Usually, the pertinent information must be developed experimentally in the laboratory concerned. Manufacturers' claims concerning parameters such as calibration stability, turn-around time and costs need to be regarded skeptically in many instances because they are often ascertained under ideal or unique conditions never found in operational practice.

C. True and False Positive and Negative Results

In qualitative testing, as in screening tests, test results are commonly reported as positive or negative for a given drug or class of drugs, depending upon the test panel of target analytes. The result is usually taken to signify that the tested specimen is positive or negative for the target analyte(s). Thus two possibilities exist for results. Two possibilities also exist with respect to the urine specimen: it either contains or does not contain the drug(s) in question. Thus, four qualitative test outcomes are possible, as shown in Table 4.

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<thead>
<tr>
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<td>True Negative</td>
</tr>
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The two true test results accurately report the factual situation and constitute the ideal, desired result. Unfortunately, practical tests are not perfect and both false negative and false positive results do occur in practice. False or incorrect results can occur because of chemical, physiological or pharmacological factors, outright errors in the analysis including instrument malfunctions and mistakes made by analysts, or inherent limitations of analysis methods. Another separate category of incorrect results is those attributable to improper reporting.

Improper reporting can occur through substitutions, transpositions or other clerical errors yielding a report which differs from the actual test result. It can also occur when a correct test result is linked with the wrong person through initial or subsequent misidentification of specimens. Analysis methods requiring substantial experience and judgment by the analyst (e.g., interpretation of a thin-layer chromatogram) are more subject to error than automated methods which make fewer demands for decision-making and judgment upon the analyst. The frequency of occurrence of false negative test results is unknown, for all practical purposes, because negative test results are rarely repeated or confirmed by further analysis. Their existence is, however, indicated by the results of proficiency testing (P.T.) in which well characterized specimens are analyzed by laboratories which will learn only after submission of their reports what the sample composition was and what the other survey participants and referee laboratories found. Even those
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IV. Technology: Drug Analysis Techniques

Although there is some overlap, it is convenient to consider the techniques used in drug analysis under the twin categories of screening tests and confirmatory analyses. Once again, space constraints limit consideration of techniques in this article to brief descriptions of their principles and some of their more relevant characteristics. Currently available and widely used screening test techniques are color and spot tests, thin-layer chromatography, and immunochemical assays. Currently available and widely used confirmatory analysis techniques are gas chromatography, high-pressure liquid chromatography, and gas chromatography/mass spectrometry. Hundreds of published variations for each of these techniques exist and the number of unpublished modifications is countless. Few, if any, laboratories use a given technique or method entirely in accordance with the originally published details. Nearly all make local modifications or changes. Supposedly, this is done to "improve" upon the original procedure, or made to adapt a given testing method to a locally available instrument or scheme or to accommodate a local laboratory situation of peculiarity. Published data concerning the characteristics of a given technique or the performance of a given method are not necessarily applicable to the local modification, nor are those characteristics determined in the laboratory at some prior time necessarily still correct for the current situation.

A. Color or Spot Tests

Early qualitative chemical tests for various elements, anions and cations, and certain compounds yielded recognizable color change or precipitation upon addition of a drop or two of various liquid reagents to a tested sample. The sample was sometimes pretreated by a simple extraction procedure or pH adjustment. Some of these tests were subsequently adapted or modified for use with urine to indicate presence of salicylates, phenothiazine tranquilizers and other drugs for emergency toxicology purposes. Key problems were applicability of available spot tests to only a few drugs - none significant in a drug-abuse context - low sensitivity, and high potential for both false positive and false negative results.

For drug-use testing applications, the original spot test principles have been modified, mostly to solid-state impregnated strip or spot tests. The qualitative saliva-alcohol strip test based on enzymatic oxidation of ethanol by alcohol oxidase is a current-generation example of spot or color tests. A commercial product introduced in 1987 uses a chemically impregnated test paper. Urine is subjected to a simple extraction procedure and the eluate is applied to a reagent paper. A blue-gray coloration surrounding the sample locus supposedly indicates presence of cocaine, morphine or other opiates, methadone, phencyclidine or amphetamines, but does not differentiate between them. The product literature indicates that numerous over-the-counter and prescription medications can produce false positive results.

In principle, it is possible to develop solid-state tests employing monoclonal and polyclonal antibodies and other immunochromatographic approaches with coupled color reaction using dyes to produce visible color changes upon contact with biological fluids containing very low concentrations of target drugs or their metabolites. They may appear in commercial form if the market warrants the required investment of time and money. However, at the time of this writing, color and spot tests or strip tests are not a significant element in drug-use testing, with the possible exception of saliva-alcohol tests, and those evaluated have proven unacceptable.

B. Thin-Layer Chromatography (TLC)

Chromatography is the family name for procedures which are primarily processes for the separation of essentially molecular mixtures by physical means. The first recorded work on column chromatography was published in 1892, although the use of paper as a chromatography medium was apparently proposed as early as 1861. Such separations employ a stationary and a mobile phase. The adsorbent is the stationary phase and the solvent or solvent mixture is the mobile phase. The principles of chromatography applied to "thin layers" of adsorbents were first described about 50 years ago and then first used for analytical (rather than preparative) purposes about 30 years ago.

survey results can be biased. It is common practice by licensing bodies and proficiency testing groups to penalize false positive results more than false negative results in scoring the P.T. submissions. Doubt in P.T. analysis results is thus often resolved in favor of negative reports.

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The many alternative methods all share common principles. A uniform, hard, thin layer of a finely-divided adsorbent medium such as silica gel is bonded onto glass plates, or cellulose acetate, nylon, polymers or other support materials. Today, commercially prepared TLC materials are almost universally employed. A urine or other liquid sample is subjected to drug separation procedures (e.g., liquid-liquid or solid-liquid extractions) using solvents and/or macroreticular polymers or substituted polysaccharides under controlled conditions of pH, time, temperature, etc., usually followed by elution of the preliminarily separated compounds by suitable solvents and their concentration by removal of the organic solvent phase. The elution of this process is redissolved in a small volume of organic solvent, a few microliters of which are placed on the TLC plate or other TLC medium at a predetermined position.

Other sample extracts and standards and controls containing known target analytes are placed in parallel on the same TLC medium at regular intervals. Other sample extracts and standards and controls containing known target analytes are placed on the same TLC medium at regular distances. After evaporation of the sample solvent, one end of the TLC medium (usually called a "plate" regardless of the actual support material) is placed into a layer of organic solvent(s) in a closed vessel and left there for a sufficient length of time to allow the solvent to migrate by capillary action for a predetermined distance from the "origin" line to effect sufficient spatial separation of the components of the sample. After removal of the residual developing solvents, the "plate" is inspected under visible and/or ultraviolet light before and after application of various visualizing reagents by sequentially spraying them on the plate or dipping the plate into dissolved visualizing reagents, which may produce colors more or less characteristic of drug or other compound classes.

The basic chromatographic measurement is distance. A given substance is, in principle, characterized and partially identifiable by the distance it has migrated from the origin, compared to the distance the solvent front or, less commonly, a standard marker has moved from the origin. The target analyte migrates as a more or less symmetrical spot upward across the plate. However, "tailing", an inverted tear drop or pear shape often occurs as do other alterations in drop or streak shape as the result of conditions under which the "development" occurred or because of overloading or other factors. The usual and most common approach to identification of visualized "spots" is to calculate their Rf value, defined as:

$$Rf = \frac{\text{Distance the "spot" has migrated from the origin}}{\text{Distance the solvent front has migrated from the origin}}$$

That value obviously can vary continuously from 0 to 1. A substance which travels with the solvent front will have an Rf = 1.0, while an analyte which remains fixed at the origin under the particular solvent, TLC medium and other conditions of the analysis will have an Rf = 0. A putative or tentative identification of the analyte is then made by comparing the observed TLC outcome to the previously-established characteristics of all target analytes of interest with respect to: 1) Rf; 2) appearance under short (254 nm) and long-wave (350 nm) ultraviolet light; 3) visualization or non-visualization of the spot after application of various reagents (i.e., functional group analysis); and 5) apparent metabolic patterns (parent drug and characteristic metabolites). Rf values and other characteristics are markedly affected by environmental factors (e.g., temperature and humidity) and many other variables in the analysis. It is, therefore, generally accepted practice to require an authentic sample of the suspected analyte to be "run" on the sample plate at the same time, as a comparison standard. When a large number of target analytes sought in any given single TLC chromatogram prevents simultaneous analysis of authentic standards of all of them on the same plate, a reference compound or marker can be "run" at the same time, and the relative Rf value calculated as the ratio of the distance migrated by the test spot to that of the reference substance spot (RRf).

Thin-layer chromatography is one of the oldest methods for drug testing, but is still in wide-scale use, especially for multi-drug screening programs. It offers the advantages of low equipment cost, relatively rapid analysis, and capability for determining more than one target analyte per analysis. Relatively low drug concentrations, as low as 0.3 - 1.0 micrograms per milliliter, can be detected under favorable circumstances. A recent outgrowth of TLC has been development of so-called high-performance TLC (HPTLC) which has enhanced the analytical capability of thin-layer chromatography. In HPTLC, the particle size of silica gel or other adsorbents and thickness of the absorbent layer are reduced, allowing for separation of drugs and metabolites in much shorter distances and increasing the sensitivity of the method.

43. See THIN-LAYER CHROMATOGRAPHY. A LABORATORY HANDBOOK (E. Silh, 2d ed. 1969).
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Thin-layer chromatography is one of the oldest methods for drug-use testing, but is still in wide-scale use, especially for multi-drug screening programs. It offers the advantages of low equipment cost, relatively rapid analysis, and capability for determining more than one target analyte per analysis. Relatively low drug concentrations, as low as 0.3 - 1.0 micrograms per milliliter, can be detected under favorable circumstances. A recent outgrowth of TLC has been development of so-called high-performance TLC (HPTLC) which has enhanced the analytical capability of thin-layer chromatography. In HPTLC, the particle size of silica gel or other adsorbents and thickness of the absorbent layer are reduced, allowing for separation of drugs and metabolites in much shorter distances and increasing the sensitivity of the method.
One consequence is a shorter analysis time.

There are also a number of shortcomings and disadvantages to TLC. Many factors affect the performance of the procedure and the results obtained, especially reproducibility of the RF values which are the primary identifying criteria in TLC. Above all, it requires considerable practice to recognize patterns of drug and metabolite presence by their visualized colored spots which are often atypical and usually accompanied by a profusion of other spots, streaks and artifacts produced by non-target substances and physiological sample components. All TLC methods are highly labor-intensive and completely dependent upon the skill, experience and judgment of the analyst. For successful TLC results, extensive sample preparation and pre-treatment is required.

In presenting details of a TLC procedure for certain drugs, one highly experienced toxicologist made the following statements concerning TLC interpretation:

One swallow does not indicate spring is here, and just as surely, one spot on a thin-layer chromatogram does not indicate a particular drug is present. The spot at a particular Rf with particular reactions to given spray reagents is a necessary condition for proof of identity, but it is not sufficient for positive proof. All drugs presumed to be present as a result of a spot on a chromatograph should be confirmed by other tests. Whenever the quantity of proof must be high, the TLC data must be confirmed by another method using different physical properties of the substance.44

Unlike immunoassays, thin-layer chromatography also first requires hydrolysis of drug and metabolite conjugates in biological samples — their splitting by acid or enzyme treatment from combination with glucuronic acid and other coupled endogenous substances for the detection of drugs, such as morphine, which are excreted in urine or carried in blood in conjugated form, if maximum analysis sensitivity is to be achieved.

Not all drugs are detectible by TLC at equal concentrations or under a single set of analytical conditions (pH of extraction, extracting solvents, developing solvents, developing time, and visualizing reagents, etc.). By altering those conditions and performing two or more TLC assays, the number of detectible target analytes and confidence in their correct identification can be increased, at additional costs of time, effort and materials. TLC is basically a qualitative separational technique; its ability to quantitate analytes is limited to rough estimates, chiefly by comparison of the unknown spot density and size with a set of standards run in parallel. Commercial TLC systems are available which include the principal materials and reagents in ready-to-use form and employ standardized manipulations for improved reproducibility of results.

Many other TLC modifications have been proposed, including two-dimensional schemes, partial automation, densitometric measurement of spot intensity for estimation of the drug concentration. None of these are currently utilized for drug-use testing.

One limitation common to all TLC techniques is the inability to readily retain the initial "raw data" output of a TLC analysis. For all practical purposes, one is limited to either preserving the actual chromatogram (i.e., the TLC "plate") which has been exposed to various chemicals and will change in appearance over time, or to photographing the TLC chromatogram at the time of the analysis, an inconvenient and secondary way of recording the analysis results. One technique which deserves wider use is removal of the TLC adsorbent region containing an unknown spot, elution of the chromatographed drug or other analyte, and analysis of the eluate by any of several instrumental means for further identification and confirmation purposes.46

The importance of independent confirmation by other appropriate means of the identity of drugs and metabolites identified tentatively by TLC cannot be overemphasized. The need for independent confirmation is well illustrated by one of the most careful and comprehensive studies of TLC applied to the identification of therapeutically significant organic bases (antihistamines, narcotics, stimulants, etc.) by Sunshine et al.46 They studied separation and identification of 138 pure drugs in nonbiological media, using 4 and 7 TLC systems (i.e., various combinations of absorbents, diluents, developing solutions, and visualizing agents). Even when using 4 of these TLC systems, they were only able to identify 113 of the 138 pure drugs, or 81.8 per cent. Using only one TLC system (e.g., their System III), 27 drugs could only be separa-

44. See Sunshine, TLC for Weak Acids, Neutrals, and Weak Bases, METHODOLOGY FOR ANALYTICAL TOXICOLOGY 412 (L. Sunshine ed. 1975).

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TLC systems (for one class of drugs - organic bases) to the probable overlap for all analytes of interest in drug-use testing, in biological sample matrices, in any one or 2 systems, even considering analytical advances. The situation is summarized succinctly by one experienced drug analyst writing on the comparable topic of Drugs and the Performance Horse.44 The drug analyst stated, "TLC simply does not have the resolving power necessary to generate a specific identification in the context of forensic chemistry."45 The same conclusions apply to high performance TLC.

The characteristics which make TLC and HPTLC unsuitable as the sole procedure for identification of drugs or drug metabolites or as confirmatory tests do make them very useful for initial screening tests. By proper selection of analysis conditions, many drugs or metabolites can be eliminated from further consideration by one or two TLC tests, instead of individual tests for all target drugs/target analytes or drug classes of interest. Phrased differently, negative results obtained by proper TLC drug-use testing are acceptable to rule out the presence of those analytes encompassed by the TLC system in use, at significant concentrations; putative or presumptive positive results require further analyses by different principles. The author's view is shared by many persons recognized in the field. A typical statement is that made by Wallace and Hamilton: "TLC and HPTLC should be viewed only as the useful screening and initial detection procedures they are, and should not be extended beyond those limits."46

A widely used TLC method for detection of abused drugs was introduced in 1968 by Davidow49 and many modifications of it have been developed. Thin-layer chromatography has since been widely used for emergency toxicological, and other drug testing and many reviews and evaluations of the technique have been published.51

47. T. Torin, Drugs and the Performance Horse 383 (1981).
48. Id. at 383.

C. Immunochemical Assays: Basic Principles

The development of a radioimmunoassay for the measurement of endogenous insulin in plasma, originally described by Yalow and Berson in the 1950-1960 period, was a sufficiently great advance to earn a share in the Nobel prize in Physiology or Medicine for Doctor Rosalyn Yalow in 1977. Immunochemical assay (often simply called immunoassay) techniques have since been successfully applied to the quantitative or semi-quantitative analysis of many compounds present in low concentrations in biological fluids, such as enzymes, hormones and drugs. In the early 1970s, techniques based on immunochemical principles were first applied to the assay of morphine and other abused drugs.

Driven by the need for rapid, sensitive, simple and selective techniques for identification and quantitation of drugs in biological fluids, preferably without the need for prior separation from the biological samples or for a pre-analysis concentration step, immunoassays rapidly found a place in drug-use testing. All immunoassays, in essence, entail the measurement of antigen-antibody reactions, which are controlled by the law of mass action, using such procedures as fluorescence or other optical measurements or radioisotopic measurements. The basic principle can be stated as:

\[
\text{Labeled Antigen} + \text{Antigen} \rightarrow \text{Labeled Antigen-Antibody Complex} + \text{Unlabeled Antibody Complex} \]

or \( Ag + Ag^* + Ab \rightleftharpoons AgAb + Ag^*Ab \), where Ag is an unlabeled

rated into 10 groups ranging from 1 to 5 in number. One can readily extrapolate statistically from the 18.2% per cent overlap rate in four TLC systems (for one class of drugs - organic bases) to the probable overlap for all analytes of interest in drug-use testing, in biological sample matrices, in any one or 2 systems, even considering analytical advances. The situation is summarized succinctly by one experienced drug analyst writing on the comparable topic of Drugs and the Performance Horse. The drug analyst stated, "TLC simply does not have the resolving power necessary to generate a specific identification in the context of forensic chemistry." The same conclusions apply to high performance TLC.

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or \[\text{Ag} + \text{Ag}^* + \text{Ab} \rightleftharpoons \text{AgAb} + \text{Ag}^*\text{Ab}, \text{where Ag is an unlabeled}\]

47. T. Tobin, Drugs and the Performance Horse 383 (1981).
48. Id. at 383.

Published by NSUWorks, 1987
antigen either in the form of a drug standard or an unknown drug substance in the specimen or control; Ag* is an antigen labeled with a radioisotope or fluorescent compound or enzyme; Ab is an antibody to the target antigen; AgAb is a complex of unlabeled antigen with its antibody; and Ag*Ab is a complex of labeled antigen with its antibody. The underlying principle is competitive protein binding. A drug in free or conjugated form in a biological sample competes for more or less specific binding sites on the antibody with labeled drug when fixed quantities of the antibody, labeled drug and test sample containing the analyte are mixed. The proportion of labeled drug molecules bound is inversely proportional to the number of unlabeled drug molecules present in the mixture. A suitable measurement is then made of the displaced labeled drug or, alternatively, of the quantity of labeled drug still bound to the antibody. Using the appropriate radioisotope signal or optical measurement, the instrument signal is compared with those of a calibration curve or other standard measurement.

The two major classifications of immunoassays are those requiring separation of the free and bound drug forms prior to the final measurement, as in radioimmunoassays, and those which do not require a separation step and are hence designated homogeneous immunoassays. As widely applied to drug-use assays at present, the former category includes radioimmunoassays (exemplified by the Abuscreen System of Roche Diagnostic Systems), while the latter includes the homogeneous enzyme immunoassay technique (exemplified by the EMIT assay of Syva Co.) and the radiative energy attenuation (REA) technique or fluorescent polarization immunoassays (exemplified by the TDx assay of Abbott Laboratories). One of the first immunoassays applied on a large scale was FRAT (Free Radical Assay Technique).\(^a\)\(^a\) It combined immunological principles with a physical analysis technique - electron spin resonance spectrometry - for the rapid examination of large numbers of biological samples in drug-use testing. Other immunoassays for drugs of abuse have been used periodically in the past and still others will undoubtedly be developed in the future.

Immunoassays share certain characteristics: High sensitivity at microgram or nanogram per milliliter concentrations and small specimen volume requirements; use of immunoglobulin antibodies produced by animals or animal cell lines and subject to variations in sensitivity and specificity and reactivity (or titer); need for little or no sample preparation or pretreatment; relative rapidity of the test; adaptability to automation and analysis of multiple specimens; and dependence on instrumentation. Another common characteristic and limitation is the discrete nature of each assay, that is, the applicability of a given immunochemical assay to only one drug or drug class (e.g., opiates or barbiturates). Repetition of the entire analysis, with different antibodies and other reagents, is thus required for each analyte of analyte class of interest. If the immunochemical assays were truly specific and responded only to a given target analyte, that one-on-one characteristic would be well worth the additional effort, cost and time required for multiple analyses for different analytes. Unfortunately, the second major characteristic of immunochemical assays in general, in addition to high sensitivity, is their cross-reactivity, that is, their response to other sample constituents that the one antigen used to produce the antibody - a property of nearly all naturally derived antibodies. This propensity, in effect, constitutes nonspecificity of the immunochemical assay for any given single drug in the presence of sufficiently high concentrations of cross-reacting substances, and can produce false-positive results. The usual situation is that the antibody (and hence the assay) is highly selective for the target analyte, which may be a given drug or a class of drugs such as the cannabinoids to all members of which the assay responds to varying degrees. This cross-reactivity within a drug class also usually produces test responses to both a parent drug and its metabolites (e.g., both morphine and morphine glucuronide). This substantially increases the sensitivity of the assay without need for pre-analysis hydrolysis of the sample. There are operational limits to the linearity of the immunoassay reactions, which can yield false negative results, as can interference by substances added to biological samples.

Because of the foregoing characteristics, immunochemical assays are very useful as screening tests for drug-use, but they uniformly require that positive results be confirmed by other non-immunochemical techniques. Obviously, screening test results, for example those obtained by immunoassays, cannot be validly confirmed by other screening tests such as thin-layer chromatography, and vice versa. The literature encompassing drug-use testing by immunoassays is extensive, and many pertinent reviews, summaries, and evaluations have been published.\(^a\)\(^a\)

52. See Dubowski, Free Radical Assay Technique for Drugs (Application to Opiate Analysis), 1 ANN. CLIN. LAB. SCI. 199 (1971).

53. See e.g. Mulé, Bastos & Jukolsky, Evaluation of Immunoassay Methods for Detection in Urine, of Drugs Subject to Abuse, 20 CLINICAL CHEMISTRY 243 (1974); IMMUNOASSAYS FOR DRUGS SUBJECT TO ABUSE (S. Mulé ed. 1974); Mulé, Whitlock & IMMUNOASSAYS FOR DRUGS SUBJECT TO ABUSE (S. Mulé ed. 1974).
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D. Enzyme Immunoassay

Enzyme immunoassay (EIA) has become a leading technique for drug-use screening tests, based on the favorable general characteristics of immunochemical assays described above, particularly the sensitivity and applicability to untreated urine specimens. The most widely used form of EIA is the Enzyme Multiplex Immunoassay Technique (EMIT) of Syva Company. EMIT is a homogeneous assay system in which the components of the reaction mixture do not need to be separated. Discrete EMIT assays are available in one or two versions (EMIT d.a.u. and EMIT st) for amphetamines, barbiturates, benzodiazepine metabolites, cannabinoids, cocaine metabolite (benzylecgonine), methadone, methaqualone, opiates, phencyclidine, and propoxyphene. Other EMIT assays are available as serum tests for various therapeutic substances and for several common toxic substances (barbiturates, benzodiazepines). Most EMIT assays for drugs of abuse are applicable only to urine specimens, but some (e.g., those for phencyclidine and benzodiazepine) are also available in a form suitable for serum specimens.

The homogeneous enzyme immunoassay (HEIA) technique uses an enzyme as the label attached to the target drug. When the enzyme-labeled drug becomes bound to an antibody raised against that drug, the activity of the enzyme is reduced. The target drug in a biological fluid sample competes with the enzyme-labeled drug for binding sites on the antibody, thereby proportionately decreasing the antibody-in-

duced inactivation of the enzyme. The enzyme activity correlates with the concentration of the target drug in the sample introduced into the reaction mixture. The activity is measured by an ultraviolet light absorbance change which is induced by the catalytic action of the enzyme on an appropriate substrate for that enzyme. In practice in current EMIT assays, urine is mixed with a reagent which contains antibodies to a particular drug together with substrate for the enzyme glucose-6-phosphate dehydrogenase (G6PDH). Binding occurs to any drug in the urine which is "recognized" by the antibody. A drug labeled with the enzyme G6PDH is then added as a second reagent. The labeled drug combines with any remaining antibody binding sites, and the enzyme activity is thereby proportionately reduced. The residual enzyme activity is directly related to the concentration of the target drug present in the urine. The active enzyme converts nicotinamide adenine dinucleotide (NAD) to its reduced form NADH, resulting in a change in ultraviolet light absorbance measured spectrophotometrically at 340 nm over a brief time span.Earlier versions of some EMIT assays, such as that for cannabinoids, used the enzyme lysosome and later the enzyme malate dehydrogenase as enzyme labels. Those enzymes occurred from natural sources in some samples and were capable of causing false positive or falsely elevated true positive results in either 2-4% or up to 10% of all urine specimens under some conditions. That possibility is somewhat reduced by the practice of performing a "blank" analysis on every positive urine sample and subtracting the "blank" reading from the test assay reading on the same sample before comparing the corrected value to the appropriate "cut-off" value for interpreting results.

It has also been found that addition of sodium chloride to yield concentrations greater than 20 grams per liter (=20mg/ml) to urine known to contain a target analyte (e.g., cannabinoids) can cause false negative results. High concentrations of other salts can also cause false negative tests, a fact known to the drug-using community. Other interferences have also been reported, as have false positive or false
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\textsuperscript{54} See Rubenstein, Schneider & Ullman, "Homogeneous" Enzyme Immunooassay. A New Immunochromatographic Technique, 47 BIOCHEM. BIOPHYS. RES. COMMUN. 846 (1972).

\textsuperscript{55} See Arcenal & Osterloh, Endogenous Lysozyme Inactivation in EMIT-dau Assays, 6 J. ANALYTICAL TOXICOLOGY 312 (1982).

\textsuperscript{56} See Kim & Cerccio, Interference by NADH with the EMIT Method of Analysis for Drugs of Abuse, 22 CLINICAL CHEMISTRY 1935 (1976); SYVA COMPANY, EMIT d.a.u. CANNABINOID URINE ASSAY, 20 (1982).
negative results in various EMIT assays.\textsuperscript{47} Substances which appear in the urine and absorb light strongly at 340 nm in an alkaline medium, can cause negative interference, as was found true for p-nitrophenol, a urinary metabolite of the pesticide parathion.\textsuperscript{48} Presence of some preservatives in urine specimens was found to inactivate the assay.\textsuperscript{49} Numerous other cross-reactions have been shown to occur, for example, in the EMIT amphetamine assay with over-the-counter products containing ephedrine, pseudoephedrine, or phenylpropanolamine.\textsuperscript{50} Specificity or selectivity of these immunoaassays is therefore an individual property which varies considerably among the several EMIT assays. It deserves emphasis that interference or cross-reactivity findings reported in the literature often pertain to earlier or different versions of these assays than those marketed currently, as illustrated by the problems encountered earlier with the lysozyme-labeled EMIT products which have been replaced by tests using other enzyme systems. However, even re-formulated immunochemical assays can be affected by non-target substances present in the biological sample. EMIT d.a.u. users were notified by Syva Company that urine from patients who had taken certain prescription and nonprescription nonsteroidal anti-inflammatory drugs (phenylpropionic acid derivatives) could cause interference with the EMIT drug abuse assays, the drugs involved being ibuprofen, fenoprofen, and naproxen.\textsuperscript{51} Next, Syva Co. notified its customers that urine from persons who had taken ibuprofen, fenoprofen, and naproxen had been found capable of affecting EMIT cannabinoid assays using malate dehydrogenase (MDH) as the enzyme label, but not the assay using G6PDH enzyme, and the fenoprofen could also yield false positive results with the EMIT assays for amphetamines, barbiturates, benzodiazepines, and methaqualone.\textsuperscript{52} Further tests demonstrated that the reformulated EMIT cannabinoid assay using G6PDH as the enzyme label introduced in July 1986 was not affected by any of the three nonsteroidal anti-inflammatory drugs mentioned above.\textsuperscript{53}The last word is never written in such matters.

The sensitivity of the several EMIT EIA assays differs among the assays and different "cutoff" concentrations are employed as the dividing line between positive and negative results. Those assays used in drug-use testing are essentially used as qualitative tests, in distinction to those used for quantitative determinations of therapeutic substances such as antiepileptic drugs. The cutoff values in urine for a given EMIT assay such as cannabinoids have been changed by the manufacturer from time to time, and different cutoff values are employed for the d.a.u. and the st test versions. Currently, the former is listed by the manufacturer as having a detection limit of 50 nanograms of 11-nor-\(\Delta^2\)-THC-9-carboxylic acid (the principal urinary metabolite of \(\Delta^2\)-THC) per ml. of urine and the latter a detection limit of 200 ng/ml.\textsuperscript{54} The respective cutoff values are 20 or 100 ng/ml for the d.a.u. test and 100 ng/ml for the st test. Other EMIT d.a.u. assays are stated by the manufacturer to have the following detection limits in urine: Amphetamines 2.0 micrograms/ml; barbiturates (as secobarbital equivalents) 2.0 micrograms/ml; benzylecgonine (cocaine metabolite) 1.6 micrograms/ml; methadone 0.5 micrograms/ml; oxazepam (benzodiazepine metabolite) 0.7 micrograms/ml; opiates (as morphine equivalents) 0.5 micrograms/ml; phencyclidine 150 nanograms/ml; propoxyphene 2.0 micrograms/ml.\textsuperscript{55} These are realistic concentrations for laboratory use of these assays.

Because of the factors discussed above under Precision, Sensitivity, and Detection Limit as analysis method characteristics, the func-

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59. See Law and Moffat, supra note 53.

60. See Baselt, supra note 57; \textit{Syva Company, EMIT d.a.u. Drug Abuse Urine Assays} (1982).

61. See Letter from D. Lorenzen, Syva Company to Syva EMIT Drug Abuse Users (Feb., 1986).


63. See Letter from D. Lorenzen, Syva Company to Customers (Apr., 1986).


negative results in various EMIT assays. Substances which appear in the urine and absorb light strongly at 340 nm in an alkaline medium, can cause negative interference, as was found true for p-nitrophenol, a urinary metabolite of the pesticide parathion. Presence of some preservatives in urine specimens was found true to inactivate the assay. Numerous other cross-reactions have been shown to occur, for example, in the EMIT amphetamine assay with over-the-counter products containing ephedrine, pseudoephedrine, or phenylpropanolamine. Specificity or selectivity of these immunosassays is therefore an individual property which varies considerably among the several EMIT assays. It deserves emphasis that interference or cross-reactivity findings reported in the literature often pertain to earlier or different versions of these assays than those marketed currently, as illustrated by the problems encountered earlier with the lysozyme-labeled EMIT products which have been replaced by tests using other enzyme systems. However, even re-formulated immunochromatographic assays can be affected by non-target substances present in the biological sample. EMIT d.a.u. users were notified by Syva Company that urine from persons who had taken certain prescription and nonprescription nonsteroidal anti-inflammatory drugs (phenylpropanolic acid derivatives) could cause interference with the EMIT drug abuse assays, the drugs involved being ibuprofen, fenoprofen, and naxopren. Next, Syva Co. notified its customers that


59. See Law and Moffat, supra note 53.

60. See Baselt, supra note 57; Syva Company, EMIT d.a.u. DRUG ABUSE Urine ASSAYS (1982).

61. See Letter from D. Lorenzen, Syva Company to Syva EMIT DRUG Abuse Assay Users (Feb., 1986).

urine from persons who had taken ibuprofen, fenoprofen, and naproxen had been found capable of affecting EMIT cannabinoid assays using malate dehydrogenase (MDH) as the enzyme label, but not the assay using G6PDH enzyme, and the fenoprofen could also yield false positive results with the EMIT assays for amphetamines, barbiturates, benzodiazepines, and methaqualone. Further tests demonstrated that the reformulated EMIT cannabinoid assay using G6PDH as the enzyme label introduced in July 1986 was not affected by any of the three nonsteroidal anti-inflammatory drugs mentioned above. The last word is never written in such matters.

The sensitivity of the several EMIT EIA assays differs among the assays and different “cutoff” concentrations are employed as the dividing line between positive and negative results. Those assays used in drug-use testing are essentially used as qualitative tests, in distinction to those used for quantitative determinations of therapeutic substances such as antiepileptic drugs. The cutoff values in urine for a given EMIT assay such as cannabinoids have been changed by the manufacturer from time to time, and different cutoff values are employed for the d.a.u. and the st test versions. Currently, the former is listed by the manufacturer as having a detection limit of 50 nanograms of 11-nor-CHE-9-carboxylic acid (the principal urinary metabolite of δ6-THC per ml of urine and the latter a detection limit of 200 ng/ml. The respective cutoff values are 20 or 100 ng/ml for the d.a.u. test and 100 ng/ml for the st test. Other EMIT d.a.u. assays are stated by the manufacturer to have the following detection limits in urine: Amphetamines 2.0 micrograms/ml; barbiturates (as secobarbital equivalents) 2.0 micrograms/ml; benzoylcegonine (cannabinoid metabolite) 1.6 micrograms/ml; methadone 0.5 micrograms/ml; oxazepam (benzodiazepine metabolite) 0.7 micrograms/ml; opiates (as morphine equivalents) 0.5 micrograms/ml; phencyclidine 150 nanograms/ml; propoxyphene 2.0 micrograms/ml. These are realistic concentrations for laboratory use of these assays.

Because of the factors discussed above under Precision, Sensitivity, and Detection Limit as analysis method characteristics, the func-
tional value selected as a "cutoff" for a given assay is different than the stated detection limit of that EMIT assay to minimize chances of obtaining false positive or false negative results. A multi-laboratory evaluation study of the EMIT d.a.u. cannabinoid assay showed generally satisfactory test performance, although some problems were noted. Of 106 urine specimens which had yielded positive results for cannabinoids with the EMIT d.a.u. test, about 35% could not be confirmed as positive for cannabinoids by a gas chromatography/mass spectrometry confirmation method (which, however, did not initially include a hydrolysis step), and 7.5% were screened negative by EMIT but yielded positive results for cannabinoids by GC/MS. EMIT assays were developed originally as positive/negative or semi-quantitative tests only. As pointed out in the manufacturer's literature (e.g., for the Cannabinoid 20 Assay), "for semiquantitative results" a standard curve for the assay can be prepared by plotting assay readings of the several standards against the respective known concentrations, but quantitative results for positive samples can only be estimated because of inherent characteristics of the assay.

E. Fluorescence Polarization Immunoassay (FPIA)

Fluorescence measurements inherently have high sensitivity, and various procedures have therefore been proposed from time to time to use spectrofluorimetry for drug-use testing. Fluorescence polarization immunoassay (FPIA) has been applied to the analysis of several major drug classes, in the form of TDx Toxicology/Abused Drug Assays (Abbott Laboratories). Discrete TDx assays are available or in development for amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine metabolite, methadone, opiates, and phencyclidine. There is also a TDx assay for ethanol which is not based on immunochemical principles, and additional discrete TDx assays are available for therapeutics such as pheobutal and tricyclic antidepressants.

By using selectively cross-reacting antibodies, the TDx Abused Drug Assays detect parent drugs and/or major metabolites within a given class of drugs with related chemical structures. The assay is homogeneous, without need for separational procedures, and depends on competitive-binding immunoassay principles, measuring the tracer by fluorescence. The principal reagents for FPIA are a fluorescent-labeled analyte (tracer) and antibodies raised against that analyte or analyte group. Blue light at 485 nm polarized to a single plane excites that tracer or fluorophore, and raises it to an excited energy state. After excitation, the fluorophore returns to steady energy state and emits green light of a different energy level and wavelength (525-550 nm). When the fluorophore is bound to the antibody, it does not rotate freely, and the emitted green light is in the same plane as the blue excitation light and thus polarization of the light is retained. Conversely, when the tracer is free to rotate because it is not bound to the antibody, the emitted green light is in a different plane than the blue excitation light, and the light is depolarized. The fluorescent tracer competes for antibody binding sites with the unlabeled analyte in the biological sample. The higher the analyte concentration, the larger the unbound tracer fraction, and the greater the decrease in polarized light fluorescence. The concentration of the analyte is inversely proportional to the degree of polarization of light. The changes in fluorescence signal corresponding to various concentration of the unlabeled analyte in the biological specimen are determined by calibration with analyte standards of known concentration. The FPIA tests for drugs of abuse can be used to show presence or absence of the analyte of interest as established by a calibrated threshold, or to quantitate the analyte concentration by use of a series of standards. The tests are designed to use urine as the biological fluid specimen.

Because the Abbott TDx Drugs of Abuse Assays are relatively new, evaluations and experience descriptions have not yet appeared in the peer-reviewed scientific literature, although the principles have been well-established and reviewed and a number of articles have been published on therapeutic drug monitoring by FPIA. A characteristic of the TDx FPIA tests is one common to competitive binding immunoassays, namely different drugs and/or metabolites within a given drug class react with the antibody to a different extent, with some additional variations in cross-reactivity depending upon the concentration of the drug involved. An example of the cross-reactivities, as determined by the manufacturer in extensive testing of each TDx assay, is

66. See Peat, supra note 53.
67. Id.
68. See SYVA COMPANY EMIT d.a.u. CANNABINOIDS 20 ASSAY (1982).

70. Per Cent Crossreactivity = (measured concentration of the drug recognized by the antibody divided by the actual concentration of the test compound) x 100.
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70. Per Cent Crossreactivity = (measured concentration of the drug recognized by the antibody divided by the actual concentration of the test compound) x 100.
as follows for the TDx barbiturates assay using secobarbital as the calibrator and for all drugs at a concentration of 0.70 micrograms/ml: 74
Butalbital = 100%; butobarbital = 147.1%; hexobarbital = 0%; pentobarbital = 90%; phenobarbital = 145.7% and; amobarbital = 140%
TDx assay sensitivities and minimum allowable threshold values reported by the manufacturer are as follows: 71

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<td>Barbiturates</td>
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</tr>
<tr>
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</tr>
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</tr>
<tr>
<td>Cocaine Metabolite</td>
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</tr>
<tr>
<td>Opiates</td>
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</tr>
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Presence of detergents in samples potentially interferes with TDx immunoassay results; sodium chloride in concentrations up to 60 grams/liter (up to 25 grams/liter for the cannabinoids assay) resulted in less than 10% error in detecting added drugs in most assays. 72 The manufacturer cautions that "confirmation should be by an equally sensitive and specific methodology using different chemical principles." 73 The manufacturer further advises "[d]ocumented procedures should be established and maintained to insure that before a result is reported as positive, that corroborating evidence exists to support that result or, in the absence of confirmation, that the result is identified as being an 'unconfirmed' result." 74

F. Radioimmunoassay

Radioimmunoassays are laboratory procedures which combine physical measurement of radioisotopes (i.e., radioactive unstable species of a chemical element) with immunological reactions between biologically produced antibodies and antigens such as drugs and drug metabolites. They have the relatively high sensitivity of immunochromatographic methods and hence small sample requirements. Furthermore, radioimmunoassays require little or no pretreatment of samples. A target drug is labeled with a radioactive isotope. The most commonly used radioactive isotopes are tritium ³H, a radionuclide with a half-life of 12.26 years which decays by beta ray emission) and iodine-125 ¹²⁵I, a radionuclide with a half-life of 60 days which decays by electron capture with gamma rays emission). Known quantities of biological specimens and radioactively-labeled drug are mixed with a known, limiting quantity of antibodies raised in animals against the target drug. The mixture is allowed to incubate, during which time that labeled drug and any unlabeled drug present in the specimen compete for binding sites on the antibody, thus reducing the fraction of radioactivity bound to the antibody. After separating the antigen (drug)-antibody complex, one can measure either the free or the bound radioactivity with a beta or gamma scintillation counter, as appropriate. Separation of the bound and unbound radioactivity is usually accomplished by precipitation of the antigen-antibody complex with a second antibody reagent, or adsorption, followed by centrifugation. The presence or absence of the target analyte is indicated by the amount of radioactivity found which is proportional to the amount of labeled drug bound to the antibody. If the supernatant fluid is counted, a positive result is indicated by free radioactivity (universally measured in counts per minute, CPM) equal to or greater than produced by a positive control containing a threshold concentration of the analyte and treated identically to an unknown specimen. If the bound radioactivity is measured, as in a pellet of precipitated and centrifuged antigen-antibody complex, presence of the analyte is indicated by radioactivity (CPM) lower than that produced by a positive control containing the threshold concentration of the analyte.

Like enzyme immunoassay and fluorescent polarization immunoassay, RIA is a procedure yielding continuous readout values. Therefore, experimentally established threshold values are used to distinguish positive from negative results. Because of the phenomenon of "nonspecific binding" (NSB), the threshold must exceed the sensitivity of the par-

https://nsuworks.nova.edu/nlr/vol11/iss2/22
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\textsuperscript{70.1} ABBOTT LABORATORIES, BARBITURATES TDx ASSAY INFORMATION (1986).

\textsuperscript{71} DIAGNOSTICS DIVISION, ABBOTT LABORATORIES, PERSONAL COMMUNICATION (Jan. 28, 1987).

\textsuperscript{72} Id.

\textsuperscript{73} ABBOTT LABORATORIES, INTRODUCTION TO TDx TOXICOLOGY/ABUSED DRUG ASSAYS (1986).

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Drug concentrations in the specimens can be determined from these calibration curves. However, in drug-use testing, RIA is most commonly used as a screening test to obtain a positive/negative result based on the pre-established "cutoff" value. The basic RIA measurement made is radioactivity in counts per minute (CPM). To enhance RIA precision and accuracy, however, counting is carried out for longer periods of time which in part depend upon the respective radioactivity of each specimen. The accumulated counts are divided by the elapsed counting time to yield average CPM data. Because the radioactivity decays over time in the isotope-labeled reagents, standards and controls must be run in parallel with the unknown specimens at the time of analysis.

Several RIA kits for drugs of abuse are currently available commercially. They include the Abuscreen System (Roche Diagnostic Systems) with separate discrete assays, using \(^{131}\)I radiolabeled antigen, for several drugs or drug classes, with the following sensitivities and cutoff values, for analysis of urine.\(^{76}\)

\(^{75}\) Logit is a mathematical function used to transform data so that a linear function is obtained; logit (x) = log (x/1-x).


\(^{77}\) See ROCHE DIAGNOSTIC SYSTEMS, ABUSCREEN SYSTEMS PACKAGE INSERT (Jan./Mar./Apr. 1986).

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Also available commercially is a Direct RIA for Urine THC Kit (Amersham Corporation) for cannabinoids in urine, using \(^{125}\)I labeled THC metabolite. Sensitivity of that assay is stated by the manufacturer as 0.8 ng/mL, with a cutoff of 10 ng/mL.\(^{78}\) Another RIA kit, using tritium (\(^{3}\)H) labeled \(\Delta^9\)-Tetrahydrocannabinol as the tracer, is available for cannabinoids quantitation in whole blood samples, the \(\Delta^9\)-THC Direct Blood RIA Kit [\(\text{H}\)] (Immunoanalysis Corporation). It can be used with whole blood, including hemolyzed samples such as those obtained postmortem. It requires a 20 microliter sample and is sensitive to 5 nanograms of \(\Delta^9\)-THC and employs a 5 ng/mL cutoff.\(^{79}\)

These RIA tests differ substantially from each other in several important characteristics including especially the cross-reactivity of their respective antibodies, as well as sensitivity of the assay. In the Abuscreen RIA for cannabinoids, using a 100 ng/mL cutoff, \(\Delta^9\)-THC (the parent drug) cross-reacts at only about 5% of the reactivity of the major urinary metabolite, 9-carboxy-THC. In the Direct RIA Cannabinoids Assay (Amersham Corporation) using a 10 ng/mL cutoff, both \(\Delta^9\)-THC 9-carboxy-THC cross-react at 100% with the antibody. This characteristic of the latter assay makes it suitable for estimating the effect of the active drug at the time a blood sample is collected while the other assays do not permit such evaluation of test results.

\(^{78}\) AMERSHAM CORPORATION, CANNABIS DIRECT RIA FOR URINE THC KIT (1985).

\(^{79}\) IMMUNOANALYSIS CORPORATION, \(\Delta^9\)-THC DIRECT BLOOD RIA KIT [\(\text{H}\)] (1986).
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These RIA tests differ substantially from each other in several important characteristics including especially the cross-reactivity of their respective antibodies, as well as sensitivity of the assay. In the Abuscreen RIA for cannabinoids, using a 100 ng/ml cutoff, Δ9-THC (the parent drug) cross-reacts at only about 5% of the reactivity of the major urinary metabolite, 9-carboxy-THC. In the Direct RIA Cannabinoids Assay (Amersham Corporation) using a 10 ng/ml cutoff, both Δ9-THC 9-carboxy-THC cross-react at 100% with the antibody. This characteristic of the latter assay makes it suitable for estimating the effect of the active drug at the time a blood sample is collected while the other assays do not permit such evaluation of test results. Δ9-THC 9-carboxy-THC cross-react at 100% with the antibody. In the Δ9-THC Direct Blood RIA [3H] test (Immunalysis Corporation), using a 5 ng/ml cutoff, Δ9-THC cross-reacts at only 0.5%. This characteristic of the latter assay makes it suitable for estimating the effect of the active drug at the time a blood sample is collected while the other assays do not permit such evaluation of test results.

78. AMERSHAM CORPORATION, CANNABIS DIRECT RIA FOR URINE THC KIT
Because of sensitivity of RIA procedures, they are not uncommon among the first, and sometimes only, methods to be developed for chemical analysis for abused drugs which occur only in low concentrations in biological fluids. Examples of such analyses are those for lysergic acid diethylamide (LSD), a hallucinogen, in urine, and for fentanyl, a potent, fast-acting narcotic analgesic used clinically in anesthesia, and its analogues in human plasma. In addition to analysis of whole blood, plasma and serum, and urine RIA has also been applied to analysis of drugs in saliva and other biological specimens.

Since about 1970 when the first application of RIA to drug-use testing took place, the technique has been widely used and the literature is extensive. Some recent evaluations of RIA have been published. Determination of Δ⁹-THC and other cannabinoids in blood and serum by RIA using both ³H and [¹³C] radioimmunoassay methods were compared with the results of gas chromatography/mass spectrometry on 100 specimens from subjects in a driving study; the 3 methods gave comparable but significantly different quantitative results and both [¹³C] RIA and GC/MS yielded 14% false positive results on whole blood quality control specimens. To increase the specificity of RIA for cannabinoids in blood and urine, an [¹³C] RIA procedure was combined with high pressure liquid chromatography for separation of the drugs and metabolites prior to RIA measurements at cannabinoids concentrations as low as 3.3 ng/ml in urine and 6.5 ng/ml in plasma. Cross-reactivity of 41 cannabinoids and non-cannabinoid phenolic constituents of cannabis to the antibody used in the Abscreen RIA for Cannabinoids was determined; the antibody was found to exhibit high selectivity for the 9-carboxy-THC metabolite while none of the non-cannabinoid phenols from the cannabis plant exhibited any cross-reactivity, and Δ⁹-THC cross-reacted at 13%. Five methods for analysis of the major urinary Δ⁹-THC metabolite were compared experimentally on urine samples from 29 subjects using Abscreen RIA, enzyme immunoassay, GC/MS, HPLC and GC/EC methods; the immunoassay methods showed higher concentrations of urinary metabolites that the other methods. The Abscreen RIA procedures for barbiturates, cocaine metabolite, opiates and phenycyclidine were routinely used for screening tests on 4355 whole blood specimens, and confirmation of positive RIA results was attempted by gas chromatography/mass spectrometry. The respective confirmation rates of the RIA assays during 1981-84 were: Cocaine/benzoylecgonine 57%; opiates 79%; phenycyclidine 49%; and barbiturates 58%. Nonspecific binding to the RIA antibodies was found and putrefaction of blood samples caused false positive RIA results. When 100 urine samples positive for cannabinoid presence by EMIT enzyme immunoassay were tested by RIA with the [¹³C] Urine THC Direct Kit (Immunalysis Corporation), results identical to those by the EMIT HEIA were obtained, with only one RIA-positive sample being negative by gas chromatography/mass spectrometry analysis. In high volume urine testing for Δ⁹-THC metabolites using EMIT d.a.u. tests with a 100 ng/ml cutoff and Abscreen RIA tests with a 100 ng/ml cutoff, the EMIT and RIA results agreed for 91% of 667 samples. The data, including gas chromatography/mass spectrometry confirmation, indicated a 4% false positive and a 10% false negative result rate for field testing with the EMIT test; and presence of the 9-carboxy-THC urinary metabolite was confirmed in 99.7% of RIA-positive urine samples (100 ng/ml cutoff) by gas chromatography/mass spectrometry using a 20 ng/ml cutoff. No fixed relationship for quantitation of cannabinoids by RIA and GC/MS was found.

82. See Cross, supra note 24.
87. Spiehler & Sedgwick, Radioimmunoassay Screening and GC/MS Confirmation of Whole Blood Samples for Drugs of Abuse, 9 J. ANALYTICAL TOXICOLOGY 63 (1985).
88. Vereby, Mulé, Alrazi & Lehrer, One Hundred EMIT Positive Cannabinoid Urine Samples Confirmed by BPA/TLC, RIA, and GC/MS, 10 J. ANALYTICAL TOXICOLOGY 79 (1986).
89. Abercrombie & Jewell, Evaluation of EMIT and RIA High Volume Test
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87. Spiehler & Sedwick, Radioimmunoassay Screening and GC/MS Confirmation of Whole Blood Samples for Drugs of Abuse, 9 J. ANALYTICAL TOXICOLOGY 63 (1985).
88. Verevey, Muhl, Alrazi & Lehrer, One Hundred EMIT Positive Cannabinoid Urine Samples Confirmed by BPA/TLC, RIA, and GC/MS, 10 J. ANALYTICAL TOXICOLOGY 79 (1986).
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drug-use screening tests are periodically reformulated with different reagents, and that the test performance and characteristics of current tests and testing schemes are not necessarily the same as those of earlier versions of the same brand of test.

G. Gas Chromatography

The most commonly used methods for confirmatory testing in drug-use analysis are currently gas chromatography, high performance liquid chromatography, and gas chromatography/mass spectrometry. All three basic procedures can be used to produce qualitative results merely identifying drugs and drug metabolites present in a biological specimen, or to yield quantitative results providing information on the identity and concentration of individual target analytes. Methods suitable for confirmatory testing can, of course, also be used as primary screening test methods under appropriate circumstances of case load. When they are so used, however, the need for confirmatory testing by an adequate alternate method remains. The following criteria have been proposed recently for evaluating nonquantitative assays - those tests that simply indicate the presence or absence of one or more specified target analytes in a given specimen:98

<table>
<thead>
<tr>
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<td>1. Determination of analytical sensitivity</td>
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<td>5. Evaluation of potential interferents</td>
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<td>6. Assessment of technical ease of performance</td>
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These criteria correspond closely to several of those included in Table 3; determination and publication of these characteristics for candidate

93. Id.
There has also been spirited debate between manufacturers of competing drug-use screening tests concerning the merits and validity of their respective test systems. An example of this appears in a decision of the Comptroller General of the United States, dated August 22, 1985 covering procurement of drug test systems by the Defense Logistics Agency, a U.S. Department of Defense component, relating to drug testing systems based on homogeneous enzyme immunoassay ("E-method") and radioimmunoassay (R-method). The decision contains the following statement:

DLA responds that this procurement was limited to drug testing systems employing the R-method because systems employing the E-method are not reliable. As evidence, it points to a quality control report prepared by the Armed Forces Institute of Pathology for the period of January through March 1985. This report showed that CompuChem Laboratory, an outside contractor which, during this period, performed the initial drug screening tests using the E-method, had only 49.1 percent correct rate on positive blind samples, while during the same period the three military laboratories being reviewed, all of which used tests employing the R-method, had correct rates of 93.2, 99 and 99.2 percent. DLA further states that the Coast Guard procurement called for drug testing systems employing the E-method only because, prior to the procurement, the Coast Guard had purchased services from different laboratories, a great majority of which used this method, and it followed its previous experience. In actuality, DLA learned that the Coast Guard did not consider the E-method reliable because it had a 58.1 percent correct rate on positive samples in fiscal year 1984 and similarly poor results in fiscal year 1983 and the first half of fiscal year 1985, and it relied on its confirmatory testing for discharge procedures.

The reference to "positive blind samples" is to proficiency test specimens with a known added drug concentration which are submitted to a testing facility disguised as routine test specimens. This is done to assess the reporting facility's performance of the indicated tests in routine operations. As previously noted, it should be remembered that drug-use screening tests are periodically reformulated with different reagents, and that the test performance and characteristics of current tests and testing schemes are not necessarily the same as those of earlier versions of the same brand of test.

G. Gas Chromatography

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Table 5. Criteria for Technical Assessment of Nonquantitative Assay Techniques

- Determination of analytical sensitivity
- Determination of imprecision near limits of detection
- Validation of analytical specificity
- Validation of accuracy over a wide range of analyte concentrations
- Evaluation of potential interferents
- Assessment of technical ease of performance

These criteria correspond closely to several of those included in Table 3; determination and publication of these characteristics for candidate

Procedures for THC Metabolites in Urine Utilizing GC/MS Confirmation, 10

3. Id.

93. Id.
methods will allow rational selection of suitably valid analysis methods and recognition of any limitation.

Gas chromatography (GC) is also interchangeably called gas-liquid chromatography (GLC) when the separating column which is the heart of a gas chromatograph is loaded or coated with a liquid phase. Like other chromatographies, GLC is primarily a separational technique for isolating an analyte from its containing medium, and adequate sample preparation is required for biological specimens to be subjected to GLC. The basic principle of GLC is use of an inert “carrier” gas such as nitrogen or helium as the mobile phase to transport a vaporized analyte (e.g., drug or metabolite) through a glass or metal column containing a stationary liquid phase to a detector at the far end of the column which signals presence of the analyte by an electrical signal. Physical and chemical interaction of the analyte and other components of the injected sample with the column packing or coating, under the instant operating conditions, determines how long it will take that component to emerge from the column outlet and be recognized by the detector after its introduction into the gas chromatograph and into the column inlet (its “retention time”). Operational parameters such as pressure and flow rate of the carrier gas, sample inlet, column oven and detector temperatures, and chemical and electrical detector conditions are optimized and then closely controlled and monitored to achieve reproducible results. The column and the detector are the major variants in GLC. Packed columns are typically 1-2 meters long, about 2.5 mm in diameter, and often coiled or curved to fit into a small oven. The packing consists of finely divided inert granular materials such as polymers, graphitized carbon black, or diatomaceous earth as a support of large surface area, coated or “loaded” with polar or nonpolar liquids serving as the stationary phase which may be bonded to the support.

Selection of appropriate packing and liquids from among the several hundred combinations commercially available, and column preparation and conditioning are very much an art as well as science. Most laboratories now use commercially prepared columns whose performance has been well characterized and published. Because of superior resolution capability for complex mixtures, in less time and with much smaller analyte quantities, capillary columns have made substantial gains over conventional packed columns in the past several years. They consist of glass or quartz tubing of about 0.5 mm outside diameter and 0.2 to 0.4 mm internal diameter, usually about 10-50 meters long and coiled. The inside column surface is usually modified, as by etching, and then coated with a uniform thin film of partitioning liquid to form a wall-coated open tubular column or bonded with a support (e.g., microcrystals of barium chloride) to form a support-coated open tubular column.

Detectors fulfill three functions in gas chromatography: 1) they signal the presence of substances to which they are sensitive; 2) they provide electrical signals which can be made proportional, within limits, to the quantity of detectable analytes reaching the detector over a finite measured time interval; and 3) by appropriate modifications of construction and operating conditions, they can be made highly selective for certain compounds, such as those containing phosphorus or nitrogen (e.g., phenylcyclidine or cocaine) or halogens like chlorine. The four GC detectors commonly used for drug analyses are: flame ionization which responds to nearly all classes of chemical compounds; alkali flame ionization, often called a nitrogen-phosphorus (N-P) detector because of its much greater sensitivity by a response factor of almost 50,000:1 to compounds containing these elements than to the typical carbon compound lacking nitrogen or phosphorus; electron capture detectors which are sensitive to compounds with a high affinity for electrons such as pesticides containing chlorine or compounds with a carbonyl (=C=O) group such as diazepam; and mass spectrometers operated in the selected ion monitoring mode, functioning essentially as a very selective GC detector. When appropriate and necessary, the sensitivity and selectivity of detection can be increased by preparing a derivative of an analyte by chemically coupling it with such elements as fluorine or phosphorus to which some GC detectors are especially sensitive. All GC detectors respond to the presence of detectable substances by an alteration in electrical signals from a baseline condition, usually an increase, but for the EC detector a decrease, in current flow. The signals are electronically amplified and presented in quantitative terms as a function of time using electronic strip-chart recorders or electronic integrators. The typical gas chromatogram is a graph consisting, ideally, of a series of sharp symmetrical spikes departing momentarily from the baseline, each of which represents a particular component of the injected sample mixture. The primary identification is made by the “retention time” of the peak associated with a given compound of interest, measured in minutes to the nearest 1/100, from the time of injection of the sample into the GC to the appearance of the peak. To compensate for variations in analysis conditions from occasion to occasion and the associated changes in retention time, it is common practice to establish a relative retention time, that is, the retention time of the analyte in question relative to that of a reference compound included in
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the injected sample.

Even more universal for identification is the concept of the retention index which uses a homologous series of n-paraffins to provide reference points on a scale. For any given set of column stationary phase and operating conditions, the elution times of the members of the series are assumed to increase by an index of 100 for each additional methylene (CH₂=) unit. On this scale, H₂ has an index of zero, methane an index of 100, ethane of 200, and so on up to the paraffins scale. The unknown peak is compared to the reference scale, and variations in retention time thus minimized. Applications of this scheme to drug-use testing has been described.⁹⁴

Quantitation of analytes of GC is performed by comparing the magnitude of the analyze detector response, as indicated by either peak height or peak area measurements, with that of an appropriate added internal standard. When a mass spectrometer is used as the detector, the ideal internal standard is a ¹³C analogue of the drug in question. Calibration is accomplished by plotting or calculating the ratio of peak height (or peak area) of target drug to that internal standard versus concentration of the target analyte. This relationship is a straight line through zero with most detectors under proper analysis conditions and over a limited concentration range. Use of internal standards is indispensable in quantitative gas chromatography to compensate, among other factors, for unintended and uncontrolled variation in the injected sample volume. Because gas chromatography is an analysis technique requiring vapor or gas state of the analyze, sample preparation, which may be extensive, must precede the actual GC instrumental analysis. Typically, it involves extraction of the drug and metabolites of interest from the matrix specimen, such as urine, under controlled pH and other conditions, usually using liquid-liquid extractions with immiscible solvents or liquid-solid extractions in which the specimen is exposed to separating columns of solid sorbents which are then successively washed to free them of specimen remains and contaminants and stripped of retained tagged analytes with eluting solvents. Concentration of the solvent solution of analytes is usually accomplished by evaporation with heat and reduced pressure. Chemical derivatization may be necessary to make the analyte sufficiently volatile to move through a GC column at feasible operating temperatures, and to increase sensitivity and specificity of the analysis.

As with other forms of chromatography, such as TLC, gas chromatography involves substantial analyst judgment and skill in the manipulations and the interpretation of the results. Because of the universal use of electronic signal amplification or the detector response, GC is one analytical technique in which the "signal to noise" ratio is an appropriate term in describing method sensitivity. Particularly with very low concentrations of drugs or metabolites in the original specimen, it is important to differentiate clearly the detector response attributable to the analyte from the baseline "noise" fluctuations caused by instrumental conditions, column bleeding or other assay artefacts. To make such judgements, it is essential to have the full original chromatogram available for subsequent review, together with others obtained on the same occasion, rather than merely peak height or peak area values for the sample in issue, printed by electronic integrators.

Gas chromatography has high sensitivity, and can therefore be very useful in the analysis of drugs and metabolites present only in very low concentrations in body fluids. Many examples of published methods could be cited; among these are recent applications of GC to analysis of biological specimens for cocaine, fentanyl, and Δ⁹-THC. Gas-liquid chromatography with a flame ionization detector was used to detect methylecgonine, a common urinary metabolite of cocaine in man, at a urinary concentration of 1 microgram/ml.⁹⁵ Presence of cocaine was confirmed in the saliva of human subjects who had received 15-40 mg of intravenously administered cocaine; gas-liquid chromatography was performed with a nitrogen-phosphorus detector with a linear range of 5-2000 nanogram/ml and a sensitivity limit of 5 ng/ml.⁹⁶ Fentanyl, a potent fast-acting narcotic analgesic and anesthetic agent, and its analogue sufentanil were measured in human plasma by gas-liquid chromatography with a nitrogen-phosphorus detector and a packed column; the method was sensitive to 0.1 ng/ml of either compound.⁹⁷ Analysis


⁹⁶. Thompson, supra note 25.

⁹⁷. Gillespie, Gandolfi, Maiorino & Vaughan, Gas Chromatographic Determination of Fentanyl and its Analogues in Human Plasma, 5 J. ANALYTICAL TOXICOLOGY.
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96. Thompson, supra note 25.

97. Gillespie, Gandolfi, Maiorino & Vaughan, Gas Chromatographic Determination of Fentanyl and its Analogues in Human Plasma, 5 J. ANALYTICAL TOXICOLOGY
of saliva was performed for Δ⁹-THC by gas-liquid chromatography, using a coated capillary column and a 6Ni electron capture detector; the method was linear for Δ⁹-THC concentrations of 5-250 nanograms/ml with a detection limit of 1 ng/ml.

Modern gas chromatographs are sophisticated and reliable instruments. Certain aspects of GC assays, such as sample injection and detector response measurement can be automated and thus standardized. Because of its wide applicability to the analysis of many drugs, gas chromatography is an attractive and useful technique. For the analysis of volatile organic compounds, especially that of ethyl alcohol in biological samples, automated headspace gas chromatography is the method of choice. However, gas chromatography is labor intensive, requires substantial instrument upkeep and careful attention to analysis conditions, and is subject to subtle changes such as column deterioration which can imperceptibly alter the analysis results over time. Further, gas chromatograms can only be "run" one at a time, sequentially, on any one instrument. These characteristics make GC very useful for analysis of single specimens, especially when many analytes are of interest, but decrease its usefulness as a primary technique for screening tests. It must also be recognized that the retention time (or index) of a GC peak is the principal (and often only) identifying criterion yielded by the technique and relied upon by the analyst. Like the spatial Rf criterion yielded by TLC, the retention time does not yield absolute and unequivocal identification of an unknown peak, even by comparison with authentic standards, controls, and internal standards. No one knows how many dozen, hundreds, or thousands of other chemical compounds can or will have an identical retention time, peak shape, and detector response if present in the sample and amenable to the extraction procedure used. Phrased differently, the identification of a presumed analyte by typical GC indices such as retention time or retention index is distinctly an assumption. The retention time is not a unique, reproducible physical characteristic of the chromatographed component under specified analysis conditions, but rather an experimental observation which is highly dependent upon the operating con-

133 (1981).

101. Id.

The universe of possible identities of a given GC peak can be greatly reduced by several alternate approaches, at additional effort and/or time: simultaneous "splitting" of the injected sample onto two GC columns which contain different packings or coatings and hence result in significantly different retention times and altered order of emergence for a given series of analytes; chemical derivatization to yield altered chromatographic properties; trapping of GC effluents corresponding to peaks of interest and their examination by other chemical or physical means, such as infrared spectrometry or mass spectrometry. A recent review of drug-use screening by capillary column gas chromatography has been published. The reviewers concluded by suggesting "caution against assigning identities on the basis of retention behavior." A vast literature on gas-liquid chromatographic analysis of drugs has accumulated since introduction of the technique by James and Martin in 1951, and it is not feasible to address this further herein.

H. High Performance Liquid Chromatography

High performance liquid chromatography (HPLC) was originally called high-pressure liquid chromatography because its moving phase, a liquid, is typically under pressures of 0.2-15 MPa (29-2175 lb./in²). The technique is quite analogous to gas-liquid chromatography, except that the drug or other analyte passes through the separating system while undergoing equilibration between two liquid phases rather than between a gas and a liquid phase as in GLC. Again, a separational column is the heart of the system. The primary system output is also the time it requires the drug to traverse the HPLC column under a given set of solvent pressure, solvent flow rate, temperature and other operating parameters and conditions. The principal characteristic sought for a given HPLC system is its high resolution, namely unequivocal separation of a solute mixture into its individual components. HPLC is an efficient separational procedure for liquids or substances which can be dissolved in liquids to become solutes. As in other chromatographies, there is a stationary phase, the inert packing in the column, and a mobile phase, the pressurized solvent liquid. A dissolved
of saliva was performed for Δ^8-THC by gas-liquid chromatography, using a coated capillary column and a 63Ni electron capture detector; the method was linear for Δ^8-THC concentrations of 5-250 nanograms/ml with a detection limit of 1 ng/ml.  

Modern gas chromatographs are sophisticated and reliable instruments. Certain aspects of GC assays, such as sample injection and detector response measurement can be automated and thus standardized. Because of its wide applicability to the analysis of many drugs, gas chromatography is an attractive and useful technique. For the analysis of volatile organic compounds, especially that of ethyl alcohol in biological samples, automated headspace gas chromatography is the method of choice.  

However, gas chromatography is labor intensive, requires substantial instrument upkeep and careful attention to analysis conditions, and is subject to subtle changes such as column deterioration which can imperceptibly alter the analysis results over time. Further, gas chromatograms can only be "run" one at a time, sequentially, on any one instrument. These characteristics make GC very useful for the analysis of single specimens, especially when many analytes are of interest, but decrease its usefulness as a primary technique for screening tests. It must also be recognized that the retention time (or index) of a GC peak is the principal (and often only) identifying criterion yielded by the technique and relied upon by the analyst. Like the spatial criterion yielded by TLC, the retention time does not yield absolute and unequivocal identification of an unknown peak, even by comparison with authentic standards, controls, and internal standards. No one knows how many dozen, hundreds, or thousands of other chemical compounds can or will have an identical retention time, peak shape, and detector response if present in the sample and amenable to the extraction procedure used. Phrased differently, the identification of a presumed analyte by typical GC indices such as retention time or retention index is distinctly an assumption. The retention time is not a unique, reproducible physical characteristic of the chromatographed component under specified analysis conditions, but rather an experimental observation which is highly dependent upon the operating conditions and instrumental parameters of that particular analysis run.

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sample or other comparable liquid sample is forced to flow through the column under high pressure and upon arrival at the column outlet is detected by an appropriate detector. The elution time from sample injection to detector response signal is the identifying characteristic of the analyte. Under defined conditions and within certain limitations, the quantitative detector response, which can be optical or electrical, is a function of the concentration of the separated analyte causing the response. Thus, HPLC can be used as a qualitative or quantitative analysis technique. Interaction of the solute with the mobile and stationary phases can be manipulated through different choices of column packing, solvents, and operating conditions, making HPLC a very versatile separational procedure.

The essential components of a typical HPLC system are one or more pumps, an injector, a column, one or more detectors, and a readout device such as an electronic strip-chart recorder or electronic integrator. High pressures are required to move the mobile phase through the column because the stationary phase, the column packing, is composed of micron-sized \((10^4\) meter) particles which offer substantial resistance to fluid flow. As an injected sample moves from the column inlet through the column, interactions of the sample components occur with the stationary and moving phases, resulting in differential separation of the components as they elute from the column in a sequence which reflects their identity and chemical characteristics as well as those of the column and solvents. Often, changes in composition of the mobile phase are required during an analysis; and use of two or more solvents in changing proportions over time may be required, a technique called solvent gradient programming.

Optimization of the separations is achieved by fine-tuning of phase selection and manipulation of operating parameters. Four basic types of liquid chromatography are employed: 1) Separation of solute molecules based on size or shape differences, called size exclusion chromatography; 2) equilibration of solutes between mobile and stationary phases on the basis of differences in dissolving power of one phase over another, defined as partition chromatography, dependent on molecular polarity; 3) ion-exchange chromatography, interactions based on differences in electrical charge of molecules or ions; and 4) adsorption chromatography, a liquid-solid interaction of the solute components with polar surface alumina or silica gel column packings using nonpolar solvents. In theory, all molecules differ from one another in size or shape, polarity, or charge and one or another of the above four liquid chromatography approaches should be able to effect adequate separations.

The practical implementation is more difficult because several stringent requirements are placed on effective HPLC instrumentation. First, a reliable and sturdy hydraulic system is needed to pump the mobile phase into and through the column under high pressure at highly controlled, reproducible and steady rates and free from contamination. Column packings able to withstand the high pressures and the various solvents are another requirement. Lastly, detectors of adequate sensitivity and stability are needed to provide signal outputs suitable for electronic amplification and recording, with low “noise” in the absence of analytes of interest.

HPLC is marked by speed, high resolution, and high sensitivity. It also allows for methodological variations to improve separations. The most common scheme for drug analysis by adsorption chromatography uses normal phase systems in which the stationary bed is strongly polar in nature (e.g., silica) and the mobile phase is nonpolar (e.g., n-hexane or tetrahydrofuran). Separation is controlled by the competition between solute molecules and mobile phase molecules for adsorption sites on the silica, with polar groups being the most strongly attracted and polar compounds therefore being more strongly retained than non-polar ones. Reversed-phase chromatography is the exact inverse of this. The stationary bed is nonpolar (e.g., composed of hydrocarbons) while the mobile phase is a polar liquid such as water or an alcohol. With such schemes, the more nonpolar the analyte, the longer it is retained. Such maneuvers allow separation of parent drugs and their more polar metabolites.

The detector choice is wide with HPLC, and four detectors have found widespread application to drug analysis: 1) The ultraviolet-visible light detector; 2) the fluorescence detector; 3) the refractive index detector; and 4) the electrochemical detector. Only the third can be considered a universal detector, since virtually all compounds cause a change in refractive index when dissolved in a solvent. The other detectors depend upon the specific properties of the solute rather than upon changes in the bulk properties of the eluant. Hence, their use can add selectivity to the HPLC analysis. There are, of course, substantial differences among the various detectors; for example, the refractive index detector is inherently rather insensitive, being typically only 1/100 as sensitive as ultraviolet light detectors for drug analyses. Combination of HPLC separations with other techniques for readout are possible, for example, HPLC with sequential analysis by radioimmunoassay or with mass spectrometry. Preparation of biological specimens for HPLC re-
quires some pretreatment, although this can usually be simpler than that required for gas chromatography. Another advantage of HPLC compared to GLC, is that polar drugs which require derivatization for GLC systems can be analyzed by HPLC with only minimal prior manipulation, such as direct extraction of analytes.

In general, HPLC applications to drug testing have been for analysis of a specific analyte rather than as screening tests for all drugs of interest, because analysis conditions need to be selected and optimized for a given drug or drug class, such as opiates. Recent drug analysis compilations include numerous HPLC procedures for many individual drugs of abuse. Likewise, recent methodological compilations devoted to one class of drugs, the cannabinoids, include details of HPLC analyses for those drugs. Among many examples, HPLC has recently been applied to determination of Δ⁹-THC and its metabolites in urine. This was achieved by combining HPLC using an ultraviolet light detector with radio-immunoassay, and to determination of the 9-carboxy acid of Δ⁹-THC, its principal urinary metabolite, by HPLC with electrochemical detection. In the latter study, HPLC with a cutoff of 20 nanograms/ml urine confirmed presence of cannabinoids in 92.1% of 63 urine specimens which had yielded positive results by homogeneous enzyme immunoassay with an EMIT d.a.u. test at a 75 ng/ml cutoff. Four of five unconfirmed EMIT positives were negative by all three confirmatory procedures used. Benzoylecgonine, a major metabolite of cocaine, was determined in urine by HPLC, using reversed phase ion pair chromatography and an ultraviolet light detector at a 50 nanograms/ml cutoff concentration, with a 20 ng/ml lower detection limit.

103. See Cannabinoid Analysis in Physiological Fluids (J.A. Vicini ed. 1979); The Analysis of Cannabinoids in Biological Fluids, NIDA Research Monograph 42 (R. Hawkins ed. 1982).

As these studies illustrate and because of the characteristics of HPLC discussed above, it is most useful as an analysis method for a given analyte in individual biological specimens or as a confirmatory technique. Further advances in HPLC technology, such as exploitation of a diode array visible/ultraviolet light spectral detector, which allows taking a complete UV-visible spectrum at several points on an eluting peak, may allow more universal application as initial drug-use screening tests.

I. Mass Spectrometry

Mass spectrometry (M/S) as a chemical analysis technique involves the electron or chemical ionization and subsequent fragmentation of molecules, and the determination of the mass to charge ratios (m/z) and of the relative abundances of the ions which are produced. From the known chemical structure of a molecule, especially its functional groups, it is possible to predict the fragmentation pattern likely to be produced under a given set of M/S conditions. Conversely, from the fragmentation pattern, it is possible to suggest a plausible structure of the original molecule. Under appropriate conditions, the molecular weight of the analyte can be determined and forms a key item of identifying information. In many, but not all, instances an unambiguous identification of an unknown substance can be made from a single adequate mass spectrum. Mass spectrometry is thus often highly selective, if not totally specific, and is capable of achieving sensitivity to the picogram (10⁻¹² gram) level. Accordingly, mass spectrometry when combined with a suitable form of chromatography (e.g., gas chromatography or high performance liquid chromatography) is generally considered to be the most conclusive technique presently available for identifying drugs or metabolites in biological specimens. However, the instrumentation required is complicated, complex, and expensive. Furthermore, the data handling and analysis components of a M/S system can also be very costly. Both routine operation and maintenance of a mass spectrometer, and especially M/S data interpretation and trouble shooting are distinctly specialized activities calling for a high order of technical competence and skill and extensive chemical knowledge.

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Mass spectrometry can produce qualitative results, identifying the
questioned analyte, or quantitative results, identifying it and establishing its concentration in the original biological specimen. A basic mass spectrometer consists of five major components, plus a data handling system which can be simple or complex and at present is most often, in essence, a dedicated micro or minicomputer: 1) A sample inlet splitter inclusive of high vacuum pumps; 2) a sample ionization source and chamber; 3) an ion separation system usually employing either a magnetic sector or a quadrupole filter; 4) an ion detection and signal amplification device; and 5) an output display and recorder. Each component can take many forms and alternatives and is subject to limitations imposed by operating conditions such as the reduced pressure required, typically 10^4 torr, temperature, etc. Depending upon its ability to separate analytes into groups of ions of different mass to charge (m/z) ratio, a mass spectrometer can be of low, medium or high resolution. The two principal means of producing ionization for drug-use testing are electron impact ionization and chemical ionization. In the former, the vaporized sample is bombarded with a stream of high energy electrons and the excess energy absorbed causes fragmentation of the molecule to produce both positive and negative ions in predictable patterns. In the latter, the vaporized sample is mixed with a large excess of a reagent gas such as methane or isobutane and then bombarded with high energy electrons. The reagent gas undergoes preferential ionization and produces chemically reactive ion species, such as CH⁺ in the case of methane, which subsequently react with the sample molecules to produce new ions predominantly with a mass one unit greater than the original molecular weight. These quasi-molecular ions become the most prominent ion in the spectrum — the so-called base peak. Electron impact and chemical ionization techniques are complementary, and the trend is toward employing both in critical identifications. Separation of analyte ions is accomplished by one of several alternate means such as acceleration and deflection along a circular path, and ions with a particular m/z value are thus collected and their intensity and abundance are recorded.

Many different modes of operation are possible in mass spectrometry. One can record a “full scan” mass spectrum which provides a complete mass spectrum for each sample component which enters the MS inlet. If there is a sufficiently high quantity of analyte present, the complete mass spectrum will yield the most conclusive identification in a pattern essentially unique for every compound and hence often referred to as a “chemical fingerprint.” It provides data on the kind, size, and stability of structural groups in the parent molecule from presence of the parent (base) ions and dissociation fragments produced from the base molecule by the ionization process along cleavage lines which usually occur at a few weak chemical bonds. A mass spectrometer can also be operated in the selected ion monitoring mode in which the mass spectrometer monitors the ion currents at only a few peaks which are characteristic of the drug or metabolite of interest. This mode is often used as a highly selective form of gas chromatographic detector; it has much higher sensitivity than the full scan spectrum mode, but provides a correspondingly less specific pattern for identification.

In practice, all mass spectrometric operations are technical compromises. Electron impact ionization (EI), for example, provides a more complex and complete mass spectrum than chemical ionization and hence can be more useful and more certain for identification of analytes. Chemical ionization (CI), on the other hand, is often more sensitive and can thus detect and measure lower concentrations of analytes at some sacrifice in specificity because CI mass spectra contain fewer peaks and are, therefore, generally less unique than EI mass spectra. Some methods of chemical ionization are relatively selective with respect to what compounds are ionized, resulting in reduction of potential interference from other components of the original biological sample or other contaminants. Another variant, negative ion chemical ionization mass spectrometry, can form negative clusters of ions, for example (Molecule + CI) with certain classes of compounds with high electron affinity such as those detectible by electron capture gas chromatography. An increase in mass spectrometric sensitivity of several orders of magnitude over positive ion techniques is thus possible, and is particularly useful when the sample quantity is limited.

The recording and presentation of mass spectrometric analysis results and the interpretation of such data is a complex undertaking. The basic procedure is detection of ions during a scan and measurement of their intensities. The signals obtained are proportional to the number of ions (abundance) of the particular mass to energy (m/z) ratio. Interpretation involves comparison of the instant experimental data with those obtained under supposedly identical instrumental conditions with known compounds, reaching educated conclusions, based on experience, on the chemical entities which yield the kind of pattern obtained in the instant analysis. Many manipulations of data are possible with modern data storage and transformation systems and procedures, e.g., subtract-

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tion of background to yield "clean" mass spectra, reconstruction of total ion current traces as a function of time (analogous to a gas or liquid chromatogram), and weighted abundance ratio computations for various peaks and peak pairs characteristic of a target analyte.

Because mass spectrometry requires a relatively clean sample separated from undesired matrix components and in gaseous form, gas chromatography is a very useful preliminary sample separation step before mass spectrometry. The resulting instrumental combination called gas chromatography/mass spectrometry efficiently combines the separating capabilities of GC with the high sensitivity and relative specificity of mass spectrometry. The GC/MS combination is, therefore, at present the leading analysis technique combination when identity of identification is at issue. Other instrumental combinations are feasible, for example, HPLC sample cleanup and separation can be used to precede mass spectrometry, with appropriate interfacing of the two instruments to accommodate the high vacuum and solvent-dissolved conditions. It is also evident that an analysis method as sensitive as mass spectrometry places great demands upon proper sample preparation and pretreatment steps to extract the target analytes from biological specimens as completely and as cleanly as possible.

Proposals have been made to combine mass spectrometry with mass spectrometry — to perform tandem mass spectrometry (MS/MS) using mass separation in lieu of chromatographic separation, followed by a second step of mass spectral analysis. This proposal has been viable with the development of a triple quadrupole mass spectrometer capable of operating in three modes — neutral scan loss, daughter scan, and selected reaction monitoring. The system allows initial indication of presence of analytes in a given drug class, followed by acquisition of complete daughter spectra for confirmation of drug identity.

Once again, a massive technical literature exists on mass spectrometry applied to forensic science applications including drug analysis. Many of the references previously cited in this article include data obtained by mass spectrometry for a variety of drugs, including amphetamines, cannabinoids, cocaine, opiates, phencyclidine, and other drugs. A recent comprehensive review of analysis of cannabinoids in biological specimens by gas chromatography/mass spectrometry has also been published. Among other useful data, it documents lower detection limits of GC/MS assays for cannabinoids in plasma at concentrations down to 0.1 nanograms/ml for the 9-carboxyl metabolite of Δ⁹-THC and 0.2 ng/ml for Δ⁹-THC, using electron capture negative ion chemical ionization mass spectrometry. More typically, a GC/MS lower detection limit of 0.5 ng/ml for the 9-carboxyl metabolite of Δ⁹-THC in urine is found at a signal-to-noise ratio of 3:1. A practical review of the application of GC/MS assays to abused drugs in body fluids was published in 1980 and briefly, but usefully, presents the state-of-the-art at that time, most of it still pertinent. In addition to GC/MS assay of the drugs listed immediately above, that review also covers assay of diazepam and its metabolites, mescaline, methadone, methaqualone, and phencyclidine.

Because a special mythology has arisen around mass spectrometry, especially gas chromatography/mass spectrometry, some cautionary closing comments seem indicated. Mass spectrometry and its GC/MS and HPLC/MS applications are indeed powerful and effective analytical tools for drug-use testing. Their complexity is mirrored in the great demands these methods make on analysts and interpreters of the data output. These analysts and interpreters must possess high levels of skill, competence, attention to detail, experience with these techniques and the particular instrument systems in use. Furthermore, these analysts...
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109. See Broherton & Yost, Determination of Drugs in Blood Serum by Mass

110. See, e.g., Finkle, Foltz & Taylor, A Comprehensive GC-MS Reference
System for Toxicological and Biomedical Purposes, 12 J. CHROMATOGR. SCI. 98
(1974); R. Martz, DRUGS AND RELATED COMPOUNDS (1977); CRC HANDBOOK OF
MASS SPECTRA DRUGS (J. Sunshine & M. Cepis ed. 1981); ANALYSIS OF DRUGS
Mass Spectra of Compounds of Forensic Interest (R. Adney ed. 1980); CLARKE'S
ISOLATION AND IDENTIFICATION OF DRUGS (A. Moffat 2d ed. 1986).

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111. See Suzuki, supra note 20.
112. See, e.g., INTRODUCTION TO FORENSIC TOXICOLOGY, supra note 4; U.S.
DEPARTMENT OF DEFENSE, supra note 5; O'Connor & Regeni, supra note 53; Frede-
rick, supra note 89; Hannon, supra note 83; Spiehler & Sedgwick, supra note 87; Ver-
eby, supra note 88.
113. See Thompson, supra note 25; Spiehler & Sedgwick, supra note 87.
114. See Spiehler & Sedgwick, supra note 87.
115. Id.
116. See Foltz, Analysis of Cannabinoids in Physiological Specimen by Gas
Chromatography/Mass Spectrometry, ADVANCES IN TOXICOLOGY 125 (R. Baselt ed.
1984).
117. Id.
118. McBurney, Bobbie & Sepp, GC/MS and EMIT Analyses for Delta-9-Tet-
rahydrocannabinol Metabolites in Plasma and Urine of Human Subjects, 10 J.
ANALYTICAL TOXICOLOGY 59 (1986).
119. See GC/MS ASSAYS FOR ABUSED DRUGS IN BODY FLUIDS, NIDA RE-
must be aware of pitfalls and shortcomings of the techniques in general and the instant analysis in particular. The competence of the analysis, and interpreter are thus both indispensable and nonmodifiable components of every GC/MS or HPLC/MS analysis. The adequacy of such assays can only be assessed, upon subsequent review, when full and complete disclosure is made of all pertinent instrument parameters and analysis conditions, results of positive and negative control analyses contemporaneous with those of any unknown sample(s), and full mass spectra, together with the applicable chromatograms, calibration data, and related analysis details. When an analysis for drugs and/or metabolites in biological specimens is competently and properly performed in all respects by adequately experienced and competent personnel and with appropriate instrumentation in good working order, that analysis represents the pinnacle of current technology applicable to this problem. In a January 27, 1987 letter to manufacturers of devices for drugs of abuse screening tests, the Food and Drug Administration included the following statement: “All positive tests should be confirmed by an independent and more specific method. Gas chromatography/mass spectrometry (GC/MS) is the confirmatory method of choice.”

V. Technology: Quality Assurance Practices

“Quality assurance” is the umbrella term often used in laboratory practice, especially in clinical laboratories and chemical laboratories, for programs of activity designed to control the identifiable and measurable factors which can affect laboratory test results, in order to ascertain and enhance the laboratory’s performance and related aspects such as specimen collection. Two of its major components are quality control and quality assessment, and each will be briefly considered. Most of the concepts and techniques discussed below were originally developed and highly refined for clinical chemistry laboratories. Such laboratories regularly produce large amounts of data, chiefly quantitative data on the identity and concentration of designated target analytes such as normal or abnormal components of body fluids. Those activities are quite similar to drug-use testing in many regards and the pertinent quality assurance literature compiled for chemical and toxicology applications will also be largely applicable to drug-use testing.  

Quality assurance activities do entail substantial costs and efforts, but are indispensable for proper laboratory management and control. Ordinarily, about 20% of a laboratory’s total efforts should be devoted to quality assurance activities, and 10% is probably the lowest acceptable level of such activities.

A. Quality Control

Quality control encompasses all systems, techniques, and procedures used to promote and assure validity and reliability of the laboratory work output. The laboratory work output generally takes the form of information. In drug-use testing, that information is ordinarily the absence or presence, identity and concentration of commonly abused drugs and/or their metabolites in human biological fluid specimens. Effective quality control requires stringent compliance with written protocols developed in advance on all relevant aspects of laboratory involvement with the specimen, not merely the analysis method. Clearly, sampling, specimen treatment and storage, actual analytical procedures and measurements, calibration, raw data observation and recording, subsequent data treatment, and result formulation and analysis interpretation are one key set of elements of those protocols. More general details on such subjects as records and reports, maintenance of apparatus and instruments, quality control testing, internal verifications, and laboratory safety together constitute the basis of good laboratory practices (GLP) in a formal sense. GLP standards have been promulgated for some laboratories subject to Federal regulation, such as those engaged in conducting chemical studies under the Toxic Substances Control Act and regulated by the U.S. Environmental Protection Agency and those engaged in non-clinical laboratory studies of drugs under the Federal Food, Drug and Cosmetic Act and regulated by the Food and Drug Administration under its Toxicology Laboratory Monitoring Program.


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122. See ENVIRONMENTAL PROTECTION AGENCY Toxic Substances Control: Good Laboratory Practice Standards (Final Rule), Fed. Reg. 48, 53922-53944 (Nov. 29, 1983).
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Adequate documentation is the core requirement for all quality control activities, and is essential for any meaningful review of operations and performance capability. Such records can be general or specific. The former include, for example, personnel rosters with inclusive dates of employment, title, and assignment for all laboratory personnel. Such records are essential, in combination with time-off and leave data, to establish what analysts and assistants were associated with or in a position to be associated with or have access to a given specimen, if subsequent challenge requires that determination. Specific record keeping extends to details such as instrument maintenance and repair logs with brief but meaningful entries reflecting problems and actions taken in response to them, and preparation and expiration dates for all reagents, usually by appropriate labeling of all stock reagent containers. An up-to-date methods manual covering all aspects of the analysis process for any given analyte or set of analytes is indispensable. For every method in use, the documentation should include procedural details, calibrations, reagent stability, use of blanks, standards and controls, literature references to the method, performance characteristics of the type listed in Tables 3 and 5 as determined within the laboratory, and data on other relevant performance issues such as recoveries of added analytes and interferences. Changes in any of the foregoing information, such as modifications or updates, should be dated and signed by the responsible laboratory supervisors and director.

The issue of laboratory results recording is more difficult. A complete records retention system should entail retention for the usual legal retention period, commonly seven years, of all “raw” data worksheets including the first written or instrument-printed record of instrument readout values, analyst observations and measurements. Many modern instruments only yield final result values, after internal raw data treatment, and computerized data collection and treatment without permanent raw or intermediate data storage is common. Whatever original records do exist should be retained for a reasonable time period. A separate, permanent set of records should be maintained on results obtained in all internal and external proficiency testing. For all instances showing inadequate performance in such proficiency testing or in the analysis of known control specimens, the documentation should also reflect remedial or corrective actions taken and subsequent adequate performance.

One very pertinent example of such quality control records is the so-called control chart. It is basically a graphical record, over a fixed time span such as one month, of the laboratory’s results in planned repetitive (say, daily) analyses of a homogeneous, stable pool of control samples for any given analyte. By comparison of daily results with the precision data for the particular analysis, one can readily see developing trends such as drift of the result values, and can at once note excursions beyond the control limits. The latter are usually the mean of the pool specimen concentration plus and minus 2 standard deviations of the mean, constituting the limits within which the measurement values are expected to lie 95% of the time for an analysis with Gaussian distribution of deviations. Upper and lower limits of 3 standard deviations from the mean represent the 99.7% confidence level. Individual result departures from the former are warning of possible trouble, consistent or frequent departures from the former indicate problems needing attention; and result departures beyond the latter ±3 standard deviations limits signal need for immediate corrective action. Modifications of the limits on the basis of actual measurement experience with the method and incorporating routine results is permissible.

B. Quality Assessment

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There are a variety of methods which can be used for quality assessment of laboratory performance. Some of them are replicate analysis of split specimens within a given analysis run or between different analysis runs, interchange of analysts, instruments and methods or combinations of these with comparison of the results thus obtained, periodic inspections, and, of course, internal and external proficiency testing programs. The term proficiency testing (PT) has come to be applied, in clinical and forensic laboratory practice, to routine participation by a number of laboratories in a program of analyzing, on a pre-established regular calendar basis, sets of specimens whose composition and target analysis values are established by an external organization or unit and are unknown to the individual participant laboratories or unit until after their respective results have been obtained and reported.

The programs are sometimes mandated by governmental agencies as part of licensure schemes and provided free of cost to participant laboratories or, more often, are conducted on a subscription basis by or under the sponsorship of professional organizations such as the American Association for Clinical Chemistry, the College of American Pathologists, the American Association of Bioanalysts, and the Forensic Sciences Foundation. The specimens, each containing one or more target analytes, are typically mailed quarterly in sets of four to eight, with results required to be submitted by participant laboratories within one or two weeks, followed by compilation and statistical data treatment of all submitted results and distribution of these compilations, often accompanied by critiques of the analysis methods reportedly used and the results obtained.

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There are, of course, many technical considerations in developing and operating proficiency testing programs. Examples are deciding on natural versus synthetic matrices for drugs, specimen stability problems inclusive of chemical preservatives which can potentially interfere with some analysis procedures, establishment of lower drug concentration limits to separate negative from positive reports on some uniform basis, and problems of unintentional drug contaminants in a natural human urine matrix pool which includes drug-containing urine.

The sources of external proficiency testing programs for drug-use testing are diverse. Some long-standing specialized programs have been operated for blood-alcohol analysis by several states as part of their laboratory licensure activities and by the Transportation Systems Center of the U.S. Department of Transportation. The Centers for Disease Control (CDC) of the U.S. Department of Health and Human Services conducted a proficiency testing program for drugs-of-abuse screening laboratories from 1972 through 1981. The College of American Pathologists has sponsored a series of toxicology "survey" programs since 1971. Beginning originally with programs using urine specimens for drugs-of-abuse testing and serum specimens for clinical

125. An outlier can be intuitively defined as an observation in a set of observations which deviates so greatly from the rest as to arouse suspicion it was generated by a different mechanism, as judged by its failure to fit within statistically established limits.


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Because of the impact of advances and changes in analytical toxicology, availability of new and additional standards and control materials and related factors, reports of laboratory performance several years old cannot readily be extrapolated to reach valid conclusions concerning the current scene, much less the recent or current performance of any given laboratory or its individual analysts. The best source for such evaluations of the overall field and state-of-the-art is the current PT programs critiques issued after each survey by the American Association for Clinical Chemistry LIP Program, the College of American Pathologists Toxicology Resource Committee, and other proficiency test sponsors. All program subscribers receive those critiques and usually keep them on file for educational purposes. 

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129. A complication in proficiency test result assessment is that incorrect reports cannot be distinguished from errors in analysis or in interpretation. The explanation is, therefore, often advanced that an erroneous PT report merely reflected an inadvertent reporting error, such as a misplaced decimal point or incorrect identification entry by analyte number. The end result of such errors is the same - an incorrect report. There is every reason to believe that such reporting inadvertences are not limited to proficiency testing.

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Absence of this information justifiably raises the suspicion that the laboratory was not performing acceptably or did not participate in any relevant proficiency testing program. Either situation is, in itself, cause for concern. Certainly, laboratories involved in drug-use testing should be participating in such external PT programs for their evaluation and educational uses, given the ready availability and modest cost of such programs. Failure to participate successfully in available pertinent external proficiency testing programs in each laboratory specialty or subspecialty in which a laboratory offers and is approved to perform tests, for example toxicology activities such as drug-use testing, is also a violation of Federal licensure requirements for those laboratories operating in interstate commerce and regulated under the Clinical Laboratory Improvement Act of 1967 or those approved for Coverage of Services under the Social Security Administration's Medicare Program. It may also be a violation of state regulatory requirements which exist in about twenty states.

The recognized shortcomings of "open" proficiency testing on identified PT specimens have brought about suggestions that "blind" proficiency testing be instituted because of suppositions that some laboratories are not subjecting open PT samples to the same testing procedures as their routine samples. Pilot studies on blind proficiency testing of drug-use testing laboratories were carried out by the Centers for Disease Control in 1973 and 1975 and the findings compared with results on identical PT specimens openly mailed to the same laboratories. Additional blind PT studies of drug-use testing laboratories were conducted by the Centers for Disease Control in 1978, 1980, and 1981 and by a university medical center in 1976. Such studies are difficult to design, conduct and evaluate because of the need to involve one or more surrogate "client" sources to introduce the blind PT samples into the laboratory among routine specimen submissions without the tested laboratory's knowledge, and because these PT specimens must be both well characterized and not readily recognizable. Optimal study design will also provide for open PT testing of the same laboratories, at the same time, with identical but labeled PT samples, so that the respective performances on open and blind proficiency testing can be compared.

In 1985, the Centers for Disease Control published the results of the six 1973-1981 blind PT surveys mentioned above, involving the testing of a total of 53 laboratories, a sample constituting about 12% of the 450 toxicology laboratories enrolled in the CDC/NIDA proficiency testing programs operated 1972-1981 for drug-use testing laboratories mainly serving methadone treatment centers. The results of an elaborate blind proficiency testing study conducted by the CDC in 1981 and included in the above report are particularly interesting and striking. It remains the single largest relevant study on blind proficiency testing of drug-use testing laboratories, with the possible exception of routine blind proficiency testing of military laboratories. The military laboratories' results have not been published. Because of space limitations, only two sets of excerpted data are shown in Table 6 and Table 7, on the laboratory performance of 13 laboratories, serving a total of 262 methadone treatment centers, in analyzing both drug-positive and drug-negative samples from blind and open PT surveys.


132. See Mason, supra note 130; Jain, Sneath & Budd, Blind Proficiency Testing in Urine Drug Screening: The Need for an Effective Quality Control Program, 11 Analytical Toxicology 142 (1977); La Motte, Guer rant & Lewis, Comparison of Laboratory Performance with Blind and Mail-Distributed Proficiency Testing Samples, 92 Public Health Rep. 554 (1977); Boone, Hansen & Hearn, Laboratory Evaluation and Assistance Efforts: Mailed, On-Site, and Blind Proficiency Testing Surveys Conducted by The Centers for Disease Control, 72 American J. Public Health 1364 (1982).

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Table 6. Comparison of Reported Laboratory Performance
Drug-Positive and Drug-Negative Samples in Open
and Blind Proficiency Testing Surveys Conducted
by CDC in 1981.188

<table>
<thead>
<tr>
<th>Drug or Drug Class</th>
<th>Drug-Positive PT Samples</th>
<th>Drug-Negative PT Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Open Survey</td>
<td>Blind Survey</td>
</tr>
<tr>
<td>Amphetamines</td>
<td>Mean CRR</td>
<td>Mean CRR</td>
</tr>
<tr>
<td>96</td>
<td>92-100</td>
<td>31</td>
</tr>
<tr>
<td>Barbiturates</td>
<td>98</td>
<td>92-100</td>
</tr>
<tr>
<td>Cocaine</td>
<td>98</td>
<td>87-100</td>
</tr>
<tr>
<td>Codeine</td>
<td>91</td>
<td>68-100</td>
</tr>
<tr>
<td>Methadone</td>
<td>100</td>
<td>97-100</td>
</tr>
<tr>
<td>Morphine</td>
<td>89</td>
<td>69-100</td>
</tr>
</tbody>
</table>

CRR = Correct Response Rate

The average number of challenges per laboratory was 28-61 for a
given drug class and all laboratories surveyed were able to detect the
drug analytes at the minimum reporting concentrations established by
the CDC/NIDA program, as evidenced by their performance in the
openly-mailed surveys. The most striking and troubling contrasts are
those appearing in columns 2 and 4 of Table 6, and the data in column
9. For whatever reason, the overall average reported performance in
analyzing drug-positive samples was greatly inferior in the blind survey
to that in the open survey. However, the overall average reported per-
formance in analyzing drug-negative samples was only slightly inferior
in the blind survey to that in the open survey. Even so, any unrecog-
nized false-positive result is unacceptable in drug-use testing if actions
affecting individual tested subjects are to be taken on the basis of those
results. Further, substantial rates of false-negative results, such as the
0-100% rates shown in Table 6, are also unacceptable failures, in the
public interest, for a system designed to differentiate drug presence
from drug absence.

As is expectable, the CDC blind proficiency testing report188 has
been greeted with dismay, disbelief, and skepticism by the laboratory
community. However, the pattern of findings is similar for all reported
blind PT studies, in such respects as the magnitude of error rates found
by CDC and other investigators. The CDC investigators concluded on
the basis of the statistical design of the 1981 blind PT study that only
0-50% of the surveyed laboratories had demonstrated acceptable per-
formance in the analysis of drug-containing samples shown in Table 7
for each of the six drug classes tested.

Table 7. Laboratories with Acceptable Performance† in
Analyzing Drug-Positive Samples in the 1981
CDC Blind Proficiency Testing Survey.187

<table>
<thead>
<tr>
<th>Drug or Drug Class</th>
<th>Number of Laboratories</th>
<th>Per Cent of Laboratories with Acceptable Performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamines</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Barbiturates</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>Cocaine</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>Codeine</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>Methadone</td>
<td>12</td>
<td>50</td>
</tr>
<tr>
<td>Morphine</td>
<td>13</td>
<td>8</td>
</tr>
</tbody>
</table>

†Acceptable performance (with P ≥ 95%) was considered a false-
negative rate of 5% or less; unacceptable performance (with
P ≥ 90%) was considered a false-negative rate of 25% or more (both
in laboratories subjected to at least 29 positive challenges for a given
drug or drug class)

135. Id.
136. Id.
137. Id.
Table 6. Comparison of Reported Laboratory Performance

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Drug-Positive PT Sample</th>
<th>Drug-Negative PT Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Open Survey</td>
<td>Blind Survey</td>
</tr>
<tr>
<td>CRR Range</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Amphetamines</td>
<td>96</td>
<td>92-100</td>
</tr>
<tr>
<td>Barbiturates</td>
<td>98</td>
<td>92-100</td>
</tr>
<tr>
<td>Cocaine</td>
<td>98</td>
<td>87-100</td>
</tr>
<tr>
<td>Codeine</td>
<td>91</td>
<td>68-100</td>
</tr>
<tr>
<td>Methadone</td>
<td>100</td>
<td>97-100</td>
</tr>
<tr>
<td>Morphine</td>
<td>89</td>
<td>69-100</td>
</tr>
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</table>

CRR = Correct Response Rate

The average number of challenges per laboratory was 28-61 for a given drug class and all laboratories surveyed were able to detect the drug analytes at the minimum reporting concentrations established by the CDC/NIDA program, as evidenced by their performance in the openly-mailed surveys. The most striking and troubling contrasts are those appearing in columns 2 and 4 of Table 6, and the data in column 9. For whatever reason, the overall average reported performance in analyzing drug-positive samples was greatly inferior in the blind survey to that in the open survey. However, the overall average reported performance in analyzing drug-negative samples was only slightly inferior in the blind survey to that in the open survey. Even so, any unrecognized false-positive result is unacceptable in drug-use testing if actions affecting individual tested subjects are to be taken on the basis of those results. Further, substantial rates of false-negative results, such as the 0-100% rates shown in Table 6, are also unacceptable failures, in the public interest, for a system designed to differentiate drug presence from drug absence.

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136. Id.
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A possible explanation for the much higher false-negative than false-positive percentages with the open and blind PT samples is the apparent failure of CDC to include appropriate metabolites together with the corresponding parent drug in the PT specimens, causing some laboratories to render negative reports because of the absence of the expected metabolite indications on thin-layer chromatographic tests. On the open PT survey, the laboratories may also have been further influenced by the PT program policy of penalizing a false positive report more heavily than a false negative report. It is a matter of further interest and concern that little change occurred in the reporting patterns on PT samples over nearly a decade, despite the obvious advances in analytical toxicology which had occurred. It is troubling that the data in Table 6 indicated false-positive error rates from 6 to 66% in some laboratories for the six drugs or drug categories listed, on blind PT samples, and from 2 to 8% even on open PT samples.

It is difficult to escape three conclusions, based on the reported blind PT studies: 1) That routine drug-use testing was far from universally adequate and acceptable during the period covered by those studies; 2) that laboratories appear to have treated open and blind PT samples differently; and 3) that blind proficiency testing is an indispensable, practical, and objective tool for monitoring the performance of drug-use testing laboratories. At least the last two conclusions remain equally binding today. In the writer's opinion, periodic on-site inspections by trained and qualified inspectors are also a necessary part of any regulatory and accreditation scheme for drug-use testing laboratories. From the foregoing information and considerations, it is evident that even some of the most highly motivated and most conscientious laboratories and laboratorians can and do produce erroneous results on occasion. Consequently, any given routine drug-use test cannot be regarded as infallible at today's state-of-the-art and practice. Only a full program of effective and comprehensive quality assurance practices can be expected to reduce the number, frequency, and magnitude of laboratory errors. Thus, both economic and technological factors are involved in improving the practice of drug-use testing to bring routine performance closer to the best feasible testing.

VI. Significance and Interpretation of Test Results

Interpretation of the results of drug-use testing is a multi-stage process. At minimum, the process includes the following elements:

- Verifying the actual laboratory findings
- Establishing the validity of the findings.
- Determining the significance of the findings.
- Resolving inconsistent results or findings.

These activities constitute the "payoff" of the entire preceding drug-use testing process and carry with them correspondingly greater responsibility.

The interpretation of drug-use testing results differs in several key aspects from that of the more familiar clinical laboratory tests. The testing process is often arcane and can in many respects be essentially unique to the testing laboratory rather than a set of standard, widely used and understood procedures and methods with well-established characteristics. Intermediate testing stages, observations and findings in drug-use testing are often undocumented. The results are not predictably within relatively narrow physiological limits for the sex, age, and race of the tested subject, as is true in clinical laboratory testing, in which "abnormal" findings are usually well correlated with the known diagnosis and clinical picture. Most clinical chemistry test results, for example, are highly predictable. Every living person will have a serum potassium or blood pH value within relatively narrow limits, with admittedly meaningful but narrow fluctuations. That is not so in drug-use testing. The analyst cannot predict whether a urine specimen will or will not contain the drugs or drug metabolites being searched for, or other relevant (or irrelevant) drugs. The ultimate user of drug-use testing results is commonly not familiar with the testing methodology used, or with its limitations and pitfalls, or with alternative causes for certain results, or with the statistical probabilities of reaching false-negative or false-positive results by any given analysis technique or testing method.

For these reasons, and others, the proper interpretation of the results of drug-use testing requires a particular expertise which is not widely available at present. The interpreter must have proper qualifications and experience in the pertinent areas of analytical toxicology inclusive of drug-use testing. Furthermore, the interpreter must possess an adequate understanding of the physiology and clinical chemistry of biological specimens, knowledge of the pharmacology and pharmacokinetics of abused drugs, and an adequate working knowledge of the relevant forensic science and legal aspects. Few persons possess these combined qualifications, because the need for such individuals and the opportunities for such professional activities have in the past
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https://nsuworks.nova.edu/nlr/vol11/iss2/22
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A. Validity and Accuracy of the Analytical Results

It is clearly helpful in assessing the validity and reliability of drug-use testing as analytical findings to know what the laboratory can expect to find with reasonable frequency and regularity. Direct, published data on this subject are scarce and should be supplemented by the analysis experience currently being gained by laboratories engaged in such testing on a large scale. Meanwhile, the data included in a 1983 article are useful. The support laboratory of a manufacturer of drug-use testing products analyzed approximately 1000 urine specimens received between September 1983 and January 1984 from 29 clinical laboratories which submitted up to 76 consecutive urine specimens which had been found to be positive for substances other than caffeine and nicotine. The total number of drugs detected in 1000 urine specimens was 3014, and 100 different drugs were identified by thin-layer chromatography, homogeneous enzyme immunoassay and a few other procedures. Table 8 shows the 30 drugs most commonly detected in that study.

<table>
<thead>
<tr>
<th>Rank</th>
<th>Substance</th>
<th>Number of Detections</th>
<th>Number of Detections</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Nicotine</td>
<td>498</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>Benzodiazepines</td>
<td>293</td>
<td>17</td>
</tr>
<tr>
<td>3</td>
<td>Acetaminophen</td>
<td>293</td>
<td>18</td>
</tr>
<tr>
<td>4</td>
<td>Cannabinoids</td>
<td>187*</td>
<td>19</td>
</tr>
<tr>
<td>5</td>
<td>Salicylates</td>
<td>164</td>
<td>20</td>
</tr>
<tr>
<td>6</td>
<td>Caffeine</td>
<td>156</td>
<td>21</td>
</tr>
<tr>
<td>7</td>
<td>Opiates</td>
<td>129</td>
<td>22</td>
</tr>
<tr>
<td>8</td>
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<td>88</td>
<td>24</td>
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<tr>
<td>10</td>
<td>Triptyline</td>
<td>86</td>
<td>25</td>
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<tr>
<td>11</td>
<td>Phenylpropanolamine</td>
<td>76</td>
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</tr>
<tr>
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<td>Phenobarbital</td>
<td>67</td>
<td>27</td>
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<tr>
<td>13</td>
<td>Diphenhydramine/dimenhydrinate</td>
<td>59</td>
<td>28</td>
</tr>
<tr>
<td>14</td>
<td>Benzylecgonine/coacaine</td>
<td>43</td>
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</tr>
<tr>
<td>15</td>
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<td>40</td>
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<td>Flurazepam Metabolites</td>
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<tr>
<td>Phenolphthalein</td>
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*886 Urines Tested

The specimen sources, largely from clinical laboratories, and the analytical methodology employed (e.g., a lower detection limit of 25 ng/ml of urine for cannabinoids and omission of tests for ethyl alcohol) imposed some limitations and special features on these findings. However, it is interesting to compare them with the data in Table 1 supra, to match the identities and frequencies of the drugs encountered with the urine drug-test results. The 30 drugs and metabolites listed in Table 8 constitute 86.8% of total drug identifications in the above study, and the most commonly detected 50 drugs covered 95% of total identifications; cannabinoids were found in 21% of the tested urine speci-

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138. METHODOLOGY FOR ANALYTICAL TOXICOLOGY 7 (J. Sunshine ed. 1975).
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mens.\textsuperscript{142} Other studies and this author's experience in the analysis of urine specimens from trauma patients in hospital emergency rooms/trauma centers indicate that about 25-28% of those specimens will yield positive results for cannabinoids at a cutoff concentration of 25 ng/ml.\textsuperscript{143}

Quantitative result statements and the use of cutoff values to establish a threshold for the positive/negative result decision deserve separate consideration. Drug-use testing does not subscribe to the categorical "zero level" approach which has been used, for example, for deciding upon acceptability of food additives which have been shown to be capable of inducing cancer in man or experimental animals. Under that approach, there is no permissible concentration or quantity of such an additive. In an analogous drug-use testing approach, a valid and correct finding of even one molecule (or in practice the minimally detectable number of molecules) of a target drug or drug metabolite would constitute a positive result. Instead, drug-use testing employs a "bright-line" threshold of impermissible target analyte concentration as the discriminative value, often it is very arbitrarily arrived at in a given drug-testing program. For some drugs, the detectability limits and corresponding cutoff values are at present in the microgram (10\textsuperscript{-6} grams)/ml range, for others in the nanogram (10\textsuperscript{-9} grams)/ml range. By some state-of-the-art techniques, picograms (10\textsuperscript{-12} grams) or femtograms (10\textsuperscript{-15} grams) of specific analytes can be detected. These are truly needle-in-the-haystack searches. Trying to detect a target analyte at concentration of 1 microgram per milliliter (i.e., one millionth of a gram/ml) is equivalent to trying to locate one particular inch in a total distance of 15.8 miles, or to trying to isolate one particular second in a total elapsed time of 11.6 days. Detecting a target analyte at a concentration of 1 nanogram per milliliter (i.e., one billionth of a gram/ml) is equivalent to seeking one particular inch in a total distance of 15,783 miles (or about 63% of the earth's circumference), or isolating one particular second in a total elapsed time of 31.8 years. One can readily calculate the distance and time analogies for the picogram/ml (trillionth of a gram/ml) concentrations of, say, fentanyl and fentanyl analogues detectable in plasma after administration of those compounds to human subjects.\textsuperscript{143,1} They border on the incomprehensible.

\textsuperscript{142} Id.

\textsuperscript{143} See Pest, supra note 53.

\textsuperscript{143,1} Gillespie, Gas Chromatographic Determination of Fentanyl and its Analogues in Human Plasma, 5 J. ANALYTICAL TOXICOLOGY 133 (1981).

Establishing the analytical validity, i.e., correctness or accuracy, of a quantitative or semi-quantitative result or other numerical analysis outcome of a drug-use test, therefore, requires consideration not only of what it is possible to achieve under ideal analysis conditions, but also what was probable to the requisite degree of certainty under the actual conditions of the sample analysis under consideration. One reasonable approach would be to calculate the range of concentrations which 95% of a sufficiently large number (20 or more) of replicate analyses at the nominal cutoff value would have yielded. That range corresponds to the mean of those replicate results plus and minus two standard deviations of the mean, for results which follow a Gaussian distribution obtained by an analysis method with zero bias. If the analysis result of the unknown sample numerically exceeds the nominal cutoff value by at least two standard deviations and if the mean of the cutoff value replicates coincides with the nominal cutoff value, one can validly state that (1) the unknown sample concentration did exceed the results which would have been yielded by 95 out of 100 samples identical to the cutoff; (2) that the unknown sample concentration of the target analyte was, therefore, greater than the cutoff value to a 95.4% probability; and (3) that the result is positive to a 95.4% probability. Put differently, 4.6 samples of 100 thus designated as positive should properly have been called negative. If one wants statistical assurance that only 3 out of 1000 identical samples (99.7%) which would yield analysis results equal to or greater than the cutoff value have concentrations below the nominal cutoff value, the unknown analysis result must equal or exceed the nominal cutoff value by 3 standard deviations, in addition to absence of systematic error analysis bias in the test used. Hence, 3 samples of 1000 thus designated as positive by the established cutoff value criterion should really have been designated as negative. If the analysis method does have a positive or negative bias, i.e., a systematic error, the corresponding correction must first be applied to the unknown analysis result by subtracting or adding a value reflecting the analysis bias (difference between the actual target concentration and the mean result) before subtracting the value equal to two (or three) standard deviations of the mean. However, these simple projections apply only to Gaussian result distributions following the well-known bell-shaped pattern.

To illustrate application of these principles, consider the data on analysis of diazepam (a widely used and abused benzodiazepine prescription drug) in serum in a recent routine proficiency test conducted by the College of American Pathologists. Of the 193 responding par-
mens.143 Other studies and this author’s experience in the analysis of urine specimens from trauma patients in hospital emergency rooms/trauma centers indicate that about 25-28% of those specimens will yield positive results for cannabinoids at a cutoff concentration of 25 ng/ml.144

Quantitative result statements and the use of cutoff values to establish a threshold for the positive/negative result decision deserve separate consideration. Drug-use testing does not subscribe to the categorical “zero level” approach which has been used, for example, for deciding upon acceptability of food additives which have been shown to be capable of inducing cancer in man or experimental animals. Under that approach, there is no permissible concentration or quantity of such an additive. In an analogous drug-use testing approach, a valid and correct finding of even one molecule (or in practice the minimally detectable number of molecules) of a target drug or drug metabolite would constitute a positive result. Instead, drug-use testing employs a “bright-line” threshold of impermissible target analyte concentration as the discriminative value; often it is very arbitrarily arrived at in a given drug-testing program. For some drugs, the detectability limits and corresponding cutoff values are at present in the microgram (10^-6 grams)/ml range, for others in the nanogram (10^-9 grams)/ml range. By some state-of-the-art techniques, picograms (10^-12 grams) or femtograms (10^-15 grams) of specific analytes can be detected. These are truly needle-in-the-haystack searches. Trying to detect a target analyte at concentration of 1 microgram per milliliter (i.e., one millionth of a gram/ml) is equivalent to trying to locate one particular inch in a total distance of 15.8 miles, or to trying to isolate one particular second in a total elapsed time of 11.6 days. Detecting a target analyte at a concentration of 1 nanogram per milliliter (i.e., one billionth of a gram/ml) is equivalent to seeking one particular inch in a total distance of 15,783 miles (or about 63% of the earth’s circumference), or isolating one particular second in a total elapsed time of 31.8 years. One can readily calculate the distance and time analogies for the picogram/ml (trillionth of a gram/ml) concentrations of, say, fentanyl and fentanyl analogues detectible in plasma after administration of those compounds to human subjects.145 They border on the incomprehensible.

142. Id.
143. See Peat, supra note 53.

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To illustrate application of these principles, consider the data on analysis of diazepam (a widely used and abused benzodiazepine prescription drug) in serum in a recent routine proficiency test conducted by the College of American Pathologists. Of the 193 responding par-
participating laboratories. 148 (76.7%) correctly identified diazepam in the serum sample, and 77 of these laboratories reported quantitative analysis results as follows (in micrograms/ml): Mean = 0.52; S.D. of the mean = 0.19; Range = 0.2-1.1. To simplify matters, also assume that the target value (weighed-in drug concentration) was approximately 0.5 mcg/ml and that the participant mean result of 0.52 mcg/ml was the actual diazepam concentration, meaning that the analysis methods employed had no systematic error or bias at that concentration. If we then designate 0.5 mcg/ml (= 500 ng/ml) as the positive/negative cutoff value, which is feasible because diazepam is detectable in serum by several methods at concentrations of 0.3 mcg/ml or less, the reported laboratory result data yielded the following picture.

The diazepam concentration which exceeds the 0.50 mcg/ml cutoff by 2 standard deviations is 0.8 mcg/ml; and only 6 of the 77 reported quantitative results should have been called positive for diazepam with a 95.4% probability of conforming to the 0.5 mcg/ml cutoff value. The number of participants results reported as equal to or greater than 0.5 mcg/ml was 50 of the 77 reported results. The participating laboratories thus reported 77 results at less than the cutoff concentration, which would presumably have been called negative relative to a 50 mcg/ml cutoff, whereas by appropriate statistical data treatment 71 of those reported results should have led to a "negative" report for diazepam. In this example, only 35.0% of all results would have been "negative" by simple matching to the cutoff value, whereas 92.5% should have been designated as negative at the 95% confidence level. Of the 77 reported quantitative results, 57 (or 74%) were in the range of 0.4-0.6 mcg/ml, i.e., within approximately 20% of the overall mean result. That is commendable performance for clinical toxicology purposes, but does not warrant the universal or nearly-universal practice of designating as positive all samples whose instrument response in a single screening test exceeds the response of a single calibrator at the nominal cutoff concentration. Reported data for other drug tests are comparable.

These considerations call for endorsement of the requirements incorporated into the "Draft Standards for Accreditation of Laboratories Engaged in Urine Drug Testing" promulgated by the National Institute on Drug Abuse in January 1987.148 that accredited laboratories state the cutoff concentrations employed for all positive/negative result reporting and report the actual drug concentrations found by confirmatory methods for all positive results in a mandatory proficiency test program. The statistical evaluation process described above for deciding upon the analytical validity of a positive/negative result designation is comparable to the "systematic approach to detection limits" and the so-called "critical level" or "criterion of detection" described in a recent symposium on detection limits held by the American Chemical Society.144 It deserves emphasis that even in an analysis by a method which has no systematic error or bias, every result can be either higher or lower than the stated result by virtue of the random variance associated with experimental error or fluctuation of the analysis precision. The confidence interval approach discussed above, in much simplified manner, guides the positive/negative result decision making in the light of the actual performance characteristics of the test method used and also indicates the amount of uncertainty which still cannot be ruled out. If the latter is large enough to be unacceptable, different decision criteria must be used, e.g., an actual cutoff equal to the nominal cutoff concentration plus three deviations of the mean for replicate analyses at the nominal cutoff to yield a 99.7% probability of the result exceeding the nominal cutoff. For many routine decisions, confidence levels of 95% or 99% are commonly used, but these limits may be inadequate for drug-use testing results, depending upon their consequences.

Similar considerations should apply to use of Rf values for determining the identity of an analyte in thin-layer chromatography, but the actual calculation of Rf values for unknown samples and standards is rarely performed in initial drug-use testing by TLC. Instead, analysts most often simply rely upon pattern recognition by visual inspection of the chromatogram, using the position of a spot or spots yielded by the unknown sample and their appearance after one or more visualizing treatments as the criteria for putative identification. After-the-fact review of such analysis results by the interpreter is difficult and limited by the continuing changes, such as color changes and/or fading, which thin-layer chromatograms undergo. The reviewer must consider presence or absence of the expectable TLC patterns produced by the

145. Draft Standards for Accreditation of Laboratories Engaged in

147. The Confidence Interval is a range of values, the extremes of which are termed confidence limits, within which - with a specified degree of confidence - the true value of a population parameter is believed to lie.
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Tabolites of the suspected drug, the extent and degree of interference by normal physiological sample components which co-extract with the target drugs, the appearance and masking effect of incidental TLC manifestations by such irrelevant drugs as caffeine or nicotine, and many other factors. Any doubts thus arising should be resolved in favor of a negative result report, or lead to reanalysis of the unknown sample by other methods of adequate selectivity, or specificity, for the suspected analyte.

Putative confirmation of presence and identity of a target analyte in, say, a urine specimen by an alternate analysis method is a required step in drug-use testing. However, this is in itself not a guarantee of a correct final result. Documentation of the actual incidence of misidentifications and otherwise incorrect result reporting in routine testing is difficult to obtain. Hence, recourse is usually had to the results of proficiency testing to indicate such problems, with the recognized limitation that open proficiency testing generally indicates only the best performance of which participating laboratories are capable. A few findings abstracted from a 1986 Urine Toxicology Survey (i.e., proficiency test) conducted by the College of American Pathologists for voluntary subscribers are interesting and pertinent. Three synthetic urine specimens containing, in total, 13 drugs or drug metabolites and openly identified as PT specimens were sent to the participants. The number of participants responding varied from 148 to 502 for different drugs or drug categories; there were 541 subscribers to the UT Survey series. For these three samples with their 13 challenges, there were 203 false positive reports overall, many of which had reportedly been confirmed by methods using different analytical principles than the initial (screening) tests, including gas chromatography, high performance liquid chromatography, and gas chromatography/mass spectrometry. False positive reports and misidentifications within a drug group, reportedly confirmed by GC/MS, included nortryptiline and other tricyclic antidepressants, propoxyphene, quinine, butalbital, pentobarbital, pheno- barbital, glutethimide, pentazocine, methaqualone, nordiazepam, and codeine. These and other drugs such as cannabinoids were also incorrectly reported as present by participants using other confirmation techniques than GC/MS. Most false positives and misidentifications were, in fact, obtained by laboratories using TLC or commercial TLC kits. If all 502 respondents had submitted one report for each of the 13 challenges (which they did not), a total of 6,526 results would have been reported. Hence, the least false positive result percentage for this survey** would be (203/6,526) x 100 = 3.1%. Obviously, the actual proportion of false positives among the results reported is different, but its magnitude is not readily apparent because of overlaps and gaps in result reporting by participants. False negative results were more frequent than false positives, and were method-dependent, with TLC yielding the highest proportion of false negatives.

The assessment of the accuracy and validity of the analytical results relies partly upon establishing whether the several required quality control procedures accompanying the test of the unknown sample yielded satisfactory results. Negative and positive controls should yield unequivocally negative and positive results, respectively, for the target analyte. Blank results should be within pre-established limits of acceptability for the instrument response involved, e.g., non-specific RIA count, or other analysis outcome, and so spaced between unknown specimens as to assure absence of "carry over" contamination from a preceding positive sample. If the analysis is quantitative, the several calibrators should yield instrument responses or final results which are mutually consistent and are linear between the lowest and highest values for unknown specimens included in the run. Replicate analyses on the same sample should yield acceptably coincident results, e.g., within 5% of each other. Overall, there should be documentation showing all relevant test parameters to be within the allowable tolerances, and absence of recognized abnormalities of the sample or the testing system which could cause test aberrations.

Validation of findings also calls for examining the key specimen characteristics which should have been observed, measured, and recorded as a preliminary to the chemical analysis. For urine specimens, these examinations are designed to detect evidence of dilution, adulteration by additives such as salt or bleach, or decomposition. The commonly observed urine specimen characteristics are its color, appearance (transparency or cloudiness), odor; those measured routinely are its pH, and specific gravity. The findings should be within expectable physiological limits if the sample is unadulterated and suitable for analysis. The pH (a measure of the hydrogen ion concentration and hence acidity or basicity) of fresh urine normally varies from 4.8 to 8.0, the mean being about 6. Stale urine is alkaline as the result of ammoniacal fermentation or bacterial decomposition of urea. The specific gravity (i.e., ratio of the density of urine to that of water at the same temperature)

tabolites of the suspected drug, the extent and degree of interference by normal physiological sample components which co-extract with the target drugs, the appearance and masking effect of incidental TLC manifestations by such irrelevant drugs as caffeine or nicotine, and many other factors. Any doubts thus arising should be resolved in favor of a negative result report, or lead to reanalysis of the unknown sample by other methods of adequate selectivity, or specificity, for the suspected analyte.

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148. Toxicology Resource Committee Set UT-C Urine Toxicology 1986 Survey 1-6 (1986).
of random specimens of urine from subjects with normal fluid intake normally varies from 1.010 to 1.025. With deprivation of fluids, a specific gravity of 1.030 is attainable; with consumption of large volumes of fluids, a specific gravity of 1.001 can be reached. Values higher than 1.030 to 1.040, corrected for temperature, suggest presence of increased amounts of solutes, which could be physiological substances such as glucose or protein, or deliberately added adulterants such as sodium chloride. If there is doubt that the specimen is urine or is undiluted, its osmolality or creatinine concentration can be readily measured; normal urine specimens typically have an osmolality of 500-900 mOsm/kg (in the fasting morning urine) and a creatinine concentration of about 0.55 to 2.5 mg/ml with 1.0 to 1.5 mg/ml as typical men values. Thus, a urine osmolality much below 500 mOsm/kg or a urine creatinine concentration much less than 0.5 mg/ml can signify dilution of urine with water. If necessary in order to authenticate or identify a urine specimen, many other physical and chemical examinations can be performed in qualified hands. Measurement of the temperature of a urine specimen has been suggested at the time of initial collection to indicate recency of voiding and absence of dilution. Obviously, this is not a useful procedure for laboratory use on urine specimens received after collection elsewhere.

Technical details of “doping-control analyses” for the 1984 Los Angeles Olympic Games were recently published. Aspects of quality control, analytical methodology, sample validation, verification of analysis results, and interpretation of drug-use testing results are described in detail. The procedures for verification of analysis results include repetition of the complete analysis of all samples positive for presence of prohibited drugs, simultaneous recheck of the analysis data on control samples, and confirmation of all positive results by gas chromatography/mass spectrometry. Less than 2% of the 1510 samples examined for over 200 analytes were found to contain substances banned by the International Olympic Committee.

Such a low prevalence (i.e., frequency of occurrence) of drug-positive results is typical of large-scale drug-use testing programs, and it affects the predictive value of positive and negative results when the test procedures are not perfect. If sensitivity and specificity of a test

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150. See supra note 40 and accompanying text.


152. Id. See also Rorner, Fundamentals of Biostatistics 51 (2d ed. 1986).
of random specimens of urine from subjects with normal fluid intake normally varies from 1.010 to 1.025. With deprivation of fluids, a specific gravity of 1.030 is attainable; with consumption of large volumes of fluids, a specific gravity of 1.001 can be reached. Values higher than 1.030 to 1.040, corrected for temperature, suggest presence of increased amounts of solutes, which could be physiological substances such as glucose or protein, or deliberately added adulterants such as sodium chloride. If there is doubt that the specimen is urine or is uniluted, its osmolality or creatinine concentration can be readily measured; normal urine specimens typically have an osmolality of 500-90 mOsm/kg (in the fasting morning urine) and a creatinine concentration of about 0.55 to 2.5 mg/ml with 1.0 to 1.5 mg/ml as typical mean values. Thus, a urine osmolality much below 500 mOsm/kg or a urine creatinine concentration much less than 0.5 mg/ml can signify dilution of urine with water. If necessary in order to authenticate or identify a urine specimen, many other physical and chemical examinations can be performed in qualified hands. Measurement of the temperature of a urine specimen has been suggested at the time of initial collection to indicate recency of voiding and absence of dilution. Obviously, this is not a useful procedure for laboratory use on urine specimens received after collection elsewhere.

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Such a low prevalence (i.e., frequency of occurrence) of drug-positive results is typical of large-scale drug-use testing programs, and it affects the predictive value of positive and negative results when the test procedures are not perfect. If sensitivity and specificity of a test were perfect, i.e., each is 100%, all positive results would be true positives and all negative results would be true negatives. However, as documented in this article and elsewhere, test results in practice usually have sensitivities and specificities less than 100% and are thus subject to error. In a population with low prevalence of drug use, few of the tested specimens will actually contain the drug searched for and many positive test results are likely to be false-positives. The probability that a positive result is a true positive increases with increased prevalence of drug presence in the tested specimens. The effect of the statistics of sensitivity and specificity on the predictive value of a positive result for several drug-use prevalences has been recently illustrated. For a given drug-use test with 95% sensitivity and 95% specificity which is applied to specimens from a population with a 1.0 prevalence of use of the target drug, the predictive value of a positive result is only 16%; it rises to 28% for a population with a 2.0% prevalence of use of the target drug and to only 50% when the prevalence is 5.0%. Increasing the specificity at the expense of the sensitivity will proportionately reduce the predictive value of negative results. These considerations call for thoughtful and conservative interpretation of the significance of both positive and negative test results.

B. Significance of Test Results

Adequate and competently performed verification of the analytical findings will yield, in general, unequivocal test results. They can then be reported, for example, as follows (some required report elements are omitted):

- The urine specimen identified above was tested for the following drugs and/or drug metabolites as the respective cutoff concentration (criterion for positive/negative result reporting) listed.

[Alphabetical list of analytes with corresponding cutoff concentrations]

The urine specimen was found to be negative for all above-listed drugs and/or drug metabolites, based on the cutoff concentrations listed above.


150. See supra note 40 and accompanying text.


150.2. Id. See also Ronner, FUNDAMENTALS OF BIOSTATISTICS 51 (2d ed. 1986).
Initial (presumptive) testing was performed by (technique and method used) and confirmatory testing was performed by (technique and method used).

or

* The urine specimen identified above was tested for the following drugs and/or drug metabolites at the respective cutoff concentration (criterion for positive/negative results reporting) listed.

[Alphabetical list of analytes with corresponding cutoff concentrations]

The urine specimen was found to be positive for (alphabetical listing of all drugs and/or drug metabolites found present), based on the cutoff concentrations listed above.

Initial (presumptive) testing was performed by (technique and method used) and confirmatory testing was performed by (technique and method used).

The first-listed (negative) report exemplifies the simplest situation — none of the drugs and/or drug metabolites tested for were detected and the test results or urine specimens were, therefore, negative. Most, if not all, large-scale drug-use testing is carried out on batches of samples by using one of the screening test methods discussed above, and specimens thus found to yield negative initial or presumptive (screening) test results are usually not submitted to other testing. Such a testing scheme does not eliminate the presence of drugs or drug metabolites of interest, in the specimen or in the subject, with 100% certainty because of the limitations of the test methods, and because of the possible effects of physiological and pharmacological variables such as volume and pH of the biological fluid (especially urine), or drug dose route, and time of administration. It does, in essence, establish that there is no indication to pursue any further the search for the contemplated analytes in the specimen under consideration. Therefore, a negative test result can arise from any of the following situations (assuming the tested specimen did originate from the designated subject):

- The subject did not use a drug encompassed in the testing scheme, or
- The subject did use a drug encompassed in the testing scheme, but the drug was not detected in the tested specimen, because
  - the dose of the drug taken was too small, or
  - the drug was used or last used too long ago, or
  - the drug was present in too low a concentration to be detectible by the tests used because of specimen dilution from high fluid intake, or pH effects or other physiological or pharmacological factors; or was present at less than the stipulated cutoff concentration, or
- the tested specimen (e.g., urine) had been adulterated, diluted, tampered with, decomposed, or otherwise altered from its original neutral state, so as to produce negative results.

On its face, the second-listed (positive) report seems equally simple: Such a positive drug, or metabolite which a priori indicates intake of a given drug, was present in the specimen, as judged by preestablished analysis criteria, and was analytically confirmed. When an adequate and appropriate testing scheme works perfectly, such positive results will correctly reflect the presence of the drug or drugs indicated within the limitations of the testing scheme and the statistical considerations discussed above. But the extension of that finding, however valid on its face, does not ipso facto equate with knowing and voluntary use of the drug(s) indicated by the positive test results. At least three situations can account for such positive results: 1) Actual use of the drug indicated by the test; 2) inadvertent, unintended, or even unknown consumption of or exposure to the identified drug (or a drug in that category); and 3) presence in the tested specimen of a substance, other than the identified drug or drug metabolite, to which the tests responded. The last-listed situation represents either a false-positive result if the substance responsible for the result was a physiological body fluid component or other non-drug entity; or at best a misidentification if the substance responsible for the result was a drug among the target analytes but not that drug identified by the drug test in issue, because of unrecognized cross-reactivity of an immunochromatographic or other factors.

Testing which is limited to drug categories cannot ordinarily distinguish between actual drug misuse and the effects of medically indi-
Initial (presumptive) testing was performed by (technique and method used) and confirmatory testing was performed by (technique and method used).

or

- The urine specimen identified above was tested for the following drugs and/or drug metabolites at the respective cutoff concentration (criterion for positive/negative results reporting) listed.

[Alphabetical list of analytes with corresponding cutoff concentrations]

The urine specimen was found to be positive for (alphabetical listing of all drugs and/or drug metabolites found present), based on the cutoff concentrations listed above.

Initial (presumptive) testing was performed by (technique and method used) and confirmatory testing was performed by (technique and method used).

The first-listed (negative) report exemplifies the simplest situation — none of the drugs and/or drug metabolites tested were detected and the test results or urine specimens were, therefore, negative. Not, if not all, large-scale drug-use testing is carried out on batches of samples by using one of the screening test methods discussed above, and specimens thus found to yield negative initial or presumptive (screening) test results are usually not submitted to other testing. Such a testing scheme does not eliminate the presence of drugs or drug metabolites of interest, in the specimen or in the subject, with 100% certainty because of the limitations of the test methods, and because of the possible effects of physiological and pharmacological variables such as volume and pH of the biological fluid (especially urine), or drug dose, route, and time of administration. It does, in essence, establish that there is no indication to pursue any further the search for the contemplated analytes in the specimen under consideration. Therefore, a negative test result can arise from any of the following situations (assuming the tested specimen did originate from the designated subject):

- The subject did not use a drug encompassed in the testing scheme, or
- The subject did use a drug encompassed in the testing scheme, but the drug was not detected in the tested specimen, because
  - the dose of the drug taken was too small, or
  - the drug was used or last used too long ago, or
- the drug was present in too low a concentration to be detectible by the tests used because of specimen dilution from high fluid intake, or pH effects or other physiological or pharmacological factors; or was present at less than the stipulated cutoff concentration, or
- the tested specimen (e.g., urine) had been adulterated, diluted, tampered with, decomposed, or otherwise altered from its original neutral state, so as to produce negative results.

On its face, the second-listed (positive) report seems equally simple: Such and such a drug, or metabolite which a priori indicates intake of a given drug, was present in the specimen, as judged by preestablished analysis criteria, and was analytically confirmed. When an adequate and appropriate testing scheme works perfectly, such positive results will correctly reflect the presence of the drug or drugs indicated, within the limitations of the testing scheme and the statistical considerations discussed above. But the extension of that finding, however valid on its face, does not ipso facto equate with knowing and voluntary use of the drug(s) indicated by the positive test results. At least three situations can account for such positive results: 1) Actual use of the drug indicated by the test; 2) inadvertent, unintended, or even unknown consumption of or exposure to the identified drug (or a drug in that category); and 3) presence in the tested specimen of a substance, other than the identified drug or drug metabolite, to which the tests responded. The last-listed situation represents either a false-positive result if the substance responsible for the result was a physiological body fluid component or other non-drug entity; or at best a misidentification if the substance responsible for the result was a drug among the target analytes but not that drug identified by the drug test in issue, because of unrecognized cross-reactivity of an immunochromical test or other factors.

Testing which is limited to drug categories cannot ordinarily distinguish between actual drug misuse and the effects of medically indi-
cated prescription drugs in the same category. A common example is inability of initial immunochemical tests for opiates to distinguish between illicit use of heroin or morphine and prescribed use of codeine as a medical or dental analgesic. Even a validly confirmed test result for conjugated morphine can simply reflect metabolism of codeine. Other examples abound. Likewise, some assays for the amphetamines group respond unintentionally to phenylpropanolamine, a common over-the-counter decongestant, and to phentermine, a prescription anorectic drug (appetite suppressant), among others. Such possibilities underlie the recommendation that all positive initial or screening test results be appropriately confirmed before a report is rendered. Suitable specific confirmatory testing will eliminate such interference phenomena from further consideration.

The second explanation for a correct positive test result is more complex. Detectible concentrations of target analytes in urine can result from unintended drug intake or exposure, if the cutoff concentration for positive/negative results is set low enough. Four examples of such "innocent" explanations for correct positive drug-test results will be described. The most widely debated such event is "passive" inhalation of marijuana smoke containing cannabinoids by a person not actively smoking marijuana, but inhaling ambient air in a confined environment, e.g., a small automobile, in the presence of persons who are actively smoking marijuana. Since the first publication of the finding of test results positive for cannabinoids following only "passive" inhalation of marijuana smoke, a number of other investigations and studies of this phenomenon have been carried out. Substantial literature now exists on this topic, in addition to other extensive studies supported by and reported to the National Institute on Drug Abuse, the findings of which remain unpublished so far. The initial 1977 study involved a single "passive" marijuana smoker who achieved cannabinoid concentrations between 50 and 269 ng/ml in urine while living for several weeks in a closed research hospital ward in the presence of five regular heavy marijuana smokers. Measurable excretion of urinary cannabinoids began one week after the onset of the smoking period and peaked 15 days later, coincident with a "contact high" reported by the passive inhaler. Other studies have yielded varying results. Two of 80 urine samples collected in the ensuing 24 hours from nonsmoking subjects confined in a small area in the presence of four marijuana smokers yielded homogeneous enzyme immunoassay results exceeding the response of a 20 ng/ml calibrator. Concentrations of Δ⁹-tetrahydrocannabinol between 2.0 and 2.2 ng/ml were found in the plasma of a subject passively exposed to marijuana smoking, reaching a peak about 20 minutes after initiation of smoking. Urine collected up to 6 hours after passive inhalation of marijuana smoke showed presence of cannabinoids metabolites at post-exposure concentrations of 0.4 to 6.8 ng/ml; the study authors commented that "this experiment clearly demonstrates that passive inhalation of cannabis smoke, under conditions similar to those met in social cannabis use, will lead to significant urinary cannabinoid concentrations of about 5 ng/ml." In five healthy subjects who had previously never used marijuana, passive inhalation of marijuana smoke for 30 minutes in an automobile produced Δ⁹-tetrahydrocannabinol concentrations in the blood higher than 13 ng/ml in four of the subjects. Cannabinoids were also detected in the urine of these subjects by RIA and HEIA assays above 13 and 20 ng/ml, respectively. These authors concluded that "the demonstration of cannabinoids in blood or urine is not unequivocal proof of active cannabis smoking."

Five men were passively exposed under controlled conditions to sidestream smoke for 4 and 16 marijuana cigarettes (containing 2.8% Δ⁹-THC) for one hour each day for 6 consecutive days. The subjective effects produced by the 16 cigarette exposure conditions were similar to those observed after active smoking of one 2.8% Δ⁹-THC cigarette. Daily mean plasma concentrations of Δ⁹-THC ranges from 2.4 to 7.4 ng/ml.

157. Id.
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151. See Baselt, supra note 57.
157. Id.
ng/ml with an individual high of 18.8 ng/ml for the 16 cigarette conditions. HEIA cannabinoid assays of urine were positive with a 20 ng/ml cutoff for 4.6 ± 2.2 and 35.2 ± 3.8 urine specimens collected during the passive smoking exposure to 4 and 16 cigarettes, respectively. After passive inhalation exposure to 16 marijuana cigarettes, a mean of 6.5 ± 5.5 urine specimens were positive by HEIA at a 100 ng/ml cutoff.158 The authors urged caution in interpreting results of urinary cannabinoid screening tests and concluded that "with sufficient time and high marijuana smoke exposure conditions, it becomes difficult to distinguish between active smoking and passive inhalation." Although there has also been denial that "passive" smoking or inhalation of marijuana smoke can yield measurable concentrations of cannabinoids in blood and urine, the foregoing and other studies159 unequivocally document that cannabinoids can and do occur in blood and urine, in readily detectable concentrations, in subjects who have had only passive exposure to marijuana smoke. Accordingly, the possibility of passive marijuana smoke inhalation yielding a positive result for cannabinoids in blood or urine cannot be lightly dismissed, and alternative explanation for such positive results should receive appropriate consideration.

Claims are also occasionally made that positive urine cannabinoid test results were caused by inadvertent ingestion of cannabis or hashish in brownies or some other food. Several studies have established that such ingestion of marijuana or hashish can produce detectable concentrations of cannabinoids in body fluids and can produce the subjective effects of cannabis on the oral consumer of cannabis. Since the major excretory route for Δ⁹-THC in man is via the bile, differences in the metabolism, effects, and elimination patterns of cannabinoids can be expected after ingestion of cannabinoids, compared to the usual route of administration by inhalation. Oral administration of 20 mg of Δ⁹-THC in a chocolate cookie to 11 subjects produced distinctly individual patterns of cannabinoids concentrations in plasma as a function of time; some peak concentrations exceeded 10 ng/ml of plasma with peaks occurring typically about one hour after ingestion.160 After ingestion, Δ⁹-THC was detectable for more than 10 hours, and subjective "highs" were perceived by the subjects at about 120 to 240 minutes after the oral intake of Δ⁹-THC, compared to those perceived a few minutes after intravenous administration or by smoking. Early studies indicated that the effects of ingestion of Δ⁹-THC appear more slowly and last longer than those produced by smoking.161 Following oral intake of a 5 mg dose of Δ⁹-THC, urine cannabinoids concentrations in excess of 100 ng/ml were observed by HEIA tests, with a peak reached within 8-10 hours and reduction of the urine cannabinoids concentration below a 100 ng/ml cutoff within 24 hours in some subjects, while the urinary cannabinoids of others remained at or above a 100 ng/ml cutoff for more than one day.162 After ingesting a dose of 20 mg of Δ⁹-THC in the form of marijuana cooked in brownies, the urine of one of 5 subjects was positive for 2 days by HEIA testing for cannabinoids at a 100 ng/ml cutoff, and remained positive at the 20 ng/ml cutoff concentration for 5 days. Another subject who received 40 mg of Δ⁹-THC orally in this form remained above a 100 ng/ml cutoff concentration for 5 days. All of the subjects felt and demonstrated notable effects from both the 20 and 40 mg doses of Δ⁹-THC.163 It is evident that ingestion of cannabis or cannabinoids, whether recognized by or unknown to the subject, can produce significant concentrations of cannabinoids in blood plasma and urine, comparable to those which result from smoking of marijuana. Such results are, of course, not false-positives but true positive results for cannabinoids from unanticipated sources.

Intake of such opiates as heroin, morphine, and codeine results in excretion of free and conjugated morphine in the urine. However, abuse of opiates has been denied by subjects whose urine specimens yielded positive test results for opiates, and the alternate explanation offered that these results were attributable to the eating of poppy seed. Poppy seeds and poppy seed oil are commonly used in food preparation, pastries, and various breads and rolls in the United States and Southern Europe. These seeds of the opium poppy (Papaver somniferum) do

159. Id.
ng/ml with an individual high of 18.8 ng/ml for the 16 cigarette conditions. HEIA cannabinoid assays of urine were positive with a 20 ng/ml cutoff for 4.6 ± 2.2 and 35.2 ± 3.8 urine specimens collected during the passive smoking exposure to 4 and 16 cigarettes, respectively. After passive inhalation exposure to 16 marihuana cigarettes, a mean of 65.5 ± 5.5 urine specimens were positive by HEIA at a 100 ng/ml cutoff.69 The authors urged caution in interpreting results of urinary cannabinoid screening tests and concluded that "with sufficient time and high marijuana smoke exposure conditions, it becomes difficult to distinguish between active smoking and passive inhalation." Although there has been denial that "passive" smoking or inhalation of marihuana smoke can yield measurable concentrations of cannabinoids in blood and urine, the foregoing and other studies unequivocally document that cannabinoids can and do occur in blood and urine, in readily detectable concentrations, in subjects who have had only passive exposure to marihuana smoke. Accordingly, the possibility of passive marihuana smoke inhalation yielding a positive result for cannabinoids in blood or urine cannot be lightly dismissed, and alternative explanation for such positive results should receive appropriate consideration.

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contain residual opiates; the morphine content was found to range from 4 to 200 mg/kg depending upon geographical origin, processing method, age, and perhaps other factors. In one study 7 subjects ate one or two poppy seed cakes each of which contained about 5 mg of morphine. The urine was found to contain both free and conjugated morphine 3 and 15 hours after ingestion of the poppy seed cake; the total morphine concentrations in urine were 0.19 ± 0.04 mcg/ml at 3 hours and 0.08 ± 0.01 mcg/ml at 15 hours after ingestion of two poppy seed cakes. When 9 persons ate poppy seed cake or freshly ground poppy seeds, maximum total morphine concentrations in urine of approximately 18 mcg/ml was found using RIA, HEIA, GC, and GC/MS procedures for analysis. The subjects all excreted maximal concentrations of urinary morphine between 3 and 5 hours after eating the poppy seed cake; after eating poppy seeds, they reached morphine concentrations of 18 mcg/ml and approximately 0.3 mcg/ml was still detectable 60 hours after the poppy seed ingestion. Codeine as well as morphine was detected in the urine. These investigators concluded that "urinary morphine levels up to 5 mcg/ml due solely to poppy seed are realistically possible given the proper circumstances." Three or four subjects who had eaten one poppy seed bagel yielded urine screening test positive for opiates by HEIA testing with a 300 ng/ml (0.3 mcg/ml) cutoff at 4%, 5%, and 6 hours after eating the bagel. All 4 subjects yielded positive screening test results at 2%, 3, 4%, and 6 hours after eating two poppy seed bagels and one subject's urine was positive at 12 hours; all positive results were confirmed by gas chromatography. The urine morphine concentrations found in these studies after both minimal and substantial consumption of poppy seed-containing foods are at or up to 60 times higher than the typical 300 ng/ml cutoff concentration of morphine used to differentiate positive from negative results in screening tests for opiates using immunochromatographic assays. Attempts to identify opiates in urine which only originate from poppy seed ingestion have so far not been successful. These facts mandate caution in attributing even properly confirmed drug-use testing results for morphine in urine solely to the taking of morphine or other opiates in conventional drug form. They also re-emphasize the need to include the cutoff concentrations used for positive/negative result decisions for all target analytes in every drug-use test report.

Cocaine was termed in 1984 "currently the drug of greatest national concern, from a public health point of view," presumably because it has in recent years showed the highest continuing rate of increase in DAWN (emergency room data) mentions, overdose deaths, and serious clinical problems. Positive tests for cocaine or its major metabolite benzoylcegonine in urine are generally considered as indications of use of cocaine administered by the routes of nasal administration, inhalation by smoking, or parenteral administration by injection. However, positive test results for benzoylcegonine can also be produced by drinking of coca leaf tea. A product marketed in the U.S. under the trade name "Health Inca Tea" has been available in many so-called health food stores. Although its ingredients are listed as "decoxainized coca leaves," analysis of "Health Inca Tea" showed an average cocaine content of 4.5 mg per bag and analysis of "Mate de Coca" tea a mean cocaine content of 5.7 mg per bag. When one cup of tea brewed from the first-named product was consumed by a volunteer subject, benzoylcegonine was detectable in the urine by gas chromatography/mass spectrometry for 29 hours after ingestion of the tea and a maximum urine benzoylcegonine concentration of 1274 ng/ml was reached two hours after ingestion. Some immunochromatographic assays for cocaine metabolite employ benzoylcegonine concentrations of 100 or 300 ng/ml as the cutoff value. Both of those concentrations were exceeded in the urine of the coca leaf tea drinkers for more than 18 hours after ingestion, and the lower value was exceeded for more than 29 hours. These findings have been confirmed in studies by other investigators. Although "Health Inca Tea" is supposedly no longer actively marketed in the United States, these findings indicate that confirmed positive test results for cocaine metabolite in urine can be caused by

166. Fritschi, supra note 164.
167. Id.
170. Note that the initials spell "HIT."
contain residual opiates; the morphine content was found to range from 4 to 200 mg/kg depending upon geographical origin, processing method, age, and perhaps other factors.164 In one study 7 subjects ate one or two poppy seed cakes each of which contained about 5 mg of morphine. The urine was found to contain both free and conjugated morphine 3 and 15 hours after ingestion of the poppy seed cake; the total morphine concentrations in urine were 0.19 ± 0.04 mcg/ml at 3 hours and 0.08 ± 0.01 mcg/ml at 15 hours after ingestion of two poppy seed cakes.166 When 9 persons ate poppy seed cake or freshly ground poppy seeds, maximum total morphine concentrations in urine of approximately 18 mcg/ml was found using RIA, HPLC, GC, and GC/MS procedures for analysis. The subjects all excreted maximal concentrations of urinary morphine between 3 and 5 hours after eating the poppy seed cake; after eating poppy seeds, they reached morphine concentrations of 18 mcg/ml and approximately 0.3 mcg/ml was all detectible 60 hours after the poppy seed ingestion. Codeine as well as morphine was detected in the urine.167 These investigators concluded that "urinary morphine levels up to 5 mcg/ml due solely to poppy seed are realistically possible given the proper circumstances."168 Thus 4 of 25 subjects who had eaten one poppy seed bagel yielded urine screening test positive for opiates by HPLC, with a 300 ng/ml (0.3 mcg/ml) cutoff at 4, 5, 6, and 6 hours after eating the bagel. All four subjects yielded positive screening test results at 1/2, 3, 4, and 6 hours after eating two poppy seed bagels and one subject's urine was positive at 12 hours; all positive results were confirmed by gas chromatography.169 The urine morphine concentrations found in these studies after both minimal and substantial consumption of poppy seed-containing foods are at or up to 60 times higher than the typical 300 ng/ml cutoff concentration of morphine used to differentiate positive from negative results in screening tests for opiates. Using immunochromatographic assays, attempts to identify opiates in urine which only originate from poppy seed ingestion have so far not been successful. These facts mi-

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164. Fritschi & Prescott, Morphine Levels in Urine Subsequent to Poppy Seed Consumption 27 FORENSIC SCI. INTR'L 111 (1985).
166. Fritschi, supra note 164.
167. Id.
170. Note that the initials spell "HITT".
ingestion of a commercial food product.

The foregoing examples of passive or unintended exposure to commonly abused drugs, or to those drugs in unconventional form in foods, serve to reinforce the point that even accurate positive test results for a given target drug or metabolite do not invariably indicate "drug use" in the conventional sense by the tested subject. The foregoing examples of findings are, of course, true positives for the respective drugs or metabolites. It is safe to assume that comparable sources for positive drug-test results will be discovered for other drugs if diligent searches are carried out.

Apart from these true-positive test results from unrecognized or unintended sources of the drugs involved, there are also many allegations that false-positive test results represent interference in initial or confirmatory tests by normal or abnormal physiological components of the tested biological fluids, most commonly urine. Some of these alternative explanation claims are quite ingenious. However, they are usually advanced in the news media without meaningful documentation.

One such allegation is the "melanin defense" for positive cannabinoids test results. Melanins are dark pigments responsible for the dark color of human skin and of hair, feathers and fur in animals. They are produced by certain tumors. Chemically they are derivatives of indole carboxylic acids. The claim is that blacks have more melanin and melanin degradation products than Caucasians and other non-blacks and that, therefore, blacks excrete more melanin in their urine than non-blacks. One frequent witness for persons accused of marijuana use by virtue of positive urine tests for cannabinoids is quoted as claiming that such tests are biased against black persons, because according to him: "(1) blacks have more melanin, the chemical responsible for skin pigmentation, in their urine than whites; (2) melanin closely resembles the marijuana metabolite found in urine, THC-carboxylic acid — so closely, in fact, that it looks like THC even on a gas chromatograph coupled with a mass spectrometer, a highly accurate methodology known as GC/MS; (3) therefore, a drug test may be positive for a black only because he has a certain amount of melanin in his urine."

The claims of interference with tests for cannabinoids by melanin and urinary serotonin metabolites were investigated by adding 5-hydroxy-indole-3-acetic acid, 5-hydroxy-indole-2-carboxylic acid, indo-


3-acetic acid and indole-3-butyric acid in concentrations up to 40 mcg/ml to urine specimens and subjecting them to previously published RIA, GC, and GC/MS procedures for cannabinoids analysis. None of the tested compounds showed any significant cross-reactivity to cannabinoids antibodies in the Roche Abuscreen Cannabinoid Assay, nor were the gas chromatographic or gas chromatograph/mass spectrometry analyses affected by the tested compounds. The study authors concluded that "the concern that certain indole carboxylic acids, particularly melanin and serotonin metabolites, might interfere in the screening and/or confirmation of the THC-COOH in urine is totally unjustified." Urine samples obtained from black subjects who claimed not to be cannabis users, and blank urine samples to which a maximal concentration of 40 mcg/ml of the first three indole compounds listed immediately above had been added, were tested with the EMIT d.a.u. Cannabinoid 100 ng, Cannabinoid 20 ng (MDH enzyme), and Cannabinoid 20 ng (G6PDH enzyme) Assays. All tests produced results considered negative in comparison to the respective 100, 20, and 20 ng/ml cannabinoids calibrators used to establish cutoff values, and none of the urine specimens obtained from the 12 black volunteers were found to be positive for cannabinoids by these assays. The author concluded that "the relatively higher concentration of melanin compounds in the urine of black or darkly pigmented individuals will not result in false positive results when using any of the Syva EMIT d.a.u. Cannabinoid Assays." In the absence of credible affirmative evidence in the scientific literature that melanins or other indole carboxylic acids interfere with any screening or confirmatory procedure for analysis of cannabinoids in urine, and in view of the cited published information to the contrary, such interference claims should be rejected at present.

A frequently avowed purpose of drug-use testing in industry is the detection of unacceptable drug effects — principally drug-induced impairment by psychoactive drugs which presumably creates workplace hazards to the drug user, other workers, property and other elements of the workplace environment. Such testing is often initiated when drug
ingestion of a commercial food product. The foregoing examples of passive or unintended exposure to commonly abused drugs, or to those drugs in unconventional form in foods, serve to reinforce the point that even accurate positive test results for a given target drug or metabolite do not invariably indicate "drug use" in the conventional sense by the tested subject. The foregoing examples of findings are, of course, true positives for the respective drugs or metabolites. It is safe to assume that comparable sources for positive drug-test results will be discovered for other drugs if diligent searches are carried out.

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The claims of interference with tests for cannabinoids by melatn and urinary serotonin metabolites were investigated by adding 5-hydroxy-indole-3-acetic acid, 5-hydroxy-indole-2-carboxylic acid, indole-

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3-acetic acid and indole-3-butyric acid in concentrations up to 40 mcg/ml to urine specimens and subjecting them to previously published RIA, GC, and GC/MS procedures for cannabinoids analysis. None of the tested compounds showed any significant cross-reactivity to cannabinoids antibodies in the Roche Abuscreen Cannabinoid Assay, nor were the gas chromatographic or gas chromatograph/mass spectrometry analyses affected by the tested compounds.174 The study authors concluded that "the concern that certain indole carboxylic acids, particularly melanin and serotonin metabolites, might interfere in the screening and/or confirmation of the THC-COOH in urine is totally unjustified."175 Urine samples obtained from black subjects who claimed not to be cannabis users, and blank urine samples to which a maximal concentration of 40 mcg/ml of the first three indole compounds listed immediately above had been added, were tested with the EMIT d.a.u. Cannabinoid 100 ng, Cannabinoid 20 ng (MDH enzyme), and Cannabinoid 20 ng (G6PDH enzyme) Assays. All tests produced results considered negative in comparison to the respective 100, 20, and 20 ng/ml cannabinoids calibrators used to establish cutoff values, and none of the urine specimens obtained from the 12 black volunteers were found to be positive for cannabinoids by these assays.176 The author concluded that "the relatively higher concentration of melanin compounds in the urine of black or darkly pigmented individuals will not result in false positive results when using any of the Syva EMIT d.a.u. Cannabinoid Assays.177 In the absence of credible affirmative evidence in the scientific literature that melanins or other indole carboxylic acids interfere with any screening or confirmatory procedure for analysis of cannabinoids in urine, and in view of the cited published information to the contrary, such interference claims should be rejected at present.

A frequently avowed purpose of drug-use testing in industry is the detection of unacceptable drug effects — principally drug-induced impairment by psychoactive drugs which presumably creates workplace hazards to the drug user, other workers, property and other elements of the workplace environment. Such testing is often initiated when drug
involvement is recognized or reasonably suspected in association with the apparent impairment or intoxication of a worker while on the job, or when accidents occur involving personal injuries to the tested person or other parties or consequential property damage. Such accidents or events are said by some to constitute the "reasonable suspicion" of drug involvement which supposedly justifies probable-cause drug-use testing. In certain specific circumstances, such as train accidents or aviation accidents, post-accident toxicological testing may also be mandated by Federal regulations178 or other statutory authority. There are also, of course, state and Federal laws179 prohibiting the operation of motor vehicles by persons who are under the influence of alcohol or other drugs180 or whose blood or breath concentrations equal or exceed a given threshold. These laws and regulations and the situations to which they pertain are outside the scope of this article.

The following generalizations apply, with rare exceptions, to interpretation of the results of drug-use testing in the context of this article. Under the best of circumstances and with valid test results, the absence or presence and identity of those drugs for which appropriate testing has been performed can be established, in the tested specimen, with "reasonable scientific certainty" (in the legal usage sense of that term) and to some definable level of statistical probability. Presence or absence of drugs or drug metabolites in drug-use testing, as previously discussed and for practical reasons, is nearly universally not an absolute, but rather a relative pronouncement conforming to a pre-established set of decisional criteria for drug identity and concentrations.

The concentration of any drug present in a tested specimen, at the time of the analysis, can also be determined to the same extent of certainty.


180. The term "under the influence of drugs" is defined as follows in P.L. 99-570, the Anti-Drug Abuse Act of 1986 approved Oct. 27, 1986: "An individual shall be conclusively presumed to be under the influence of drugs if the quantity of the drug in the system of the individual would be sufficient to impair the perception, mental processes, or motor functions of the average individual." (Title 18 U.S.C., Chapter 18, §343).

If the appropriate analyses are performed. These are essentially factual findings of definable validity. Their significance is not equally clearcut and is greatly, or sometimes totally, dependent upon the reason for seeking the information and its contemplated use. If the mere presence or absence of any of a specified list of drugs, or their metabolites, in a given biological specimen and above significant concentration thresholds, is to be established as an announced acceptability/nonacceptability criterion in considering applicants for employment or candidates for promotion, the existing technology for drug-use testing is fully adequate and the interpretation is simple and clearcut. By expending the necessary effort, time, and money one can also establish conclusively whether any specified drug or drug metabolite is present in a biological specimen at the time of analysis at or above any presently detectable concentration.

Except for ethyl alcohol in blood, breath, or saliva, drug concentrations have not been extensively and validly correlated experimentally and in operational practice with their effects on large groups of subjects under a variety of conditions and circumstances and according to clear and pertinent criteria. Hence, it is the consensus of informed opinion that the data base is lacking, with rare exceptions, to make adequately valid judgments concerning the effects, e.g., on combined cognitive and motor functions, of a given concentration or concentration range of psychoactive drugs and/or their psychoactive metabolites in the tested body fluid, on a given person under given circumstances. Presence of other psychological, physiological, or pharmacological factors (e.g., anxiety, fatigue, hypoglycemia or other biochemical abnormality, presence of other drugs or toxic substances, etc.) adds further confounding elements to the difficulties of interpreting the drug-use test findings. Lack of information concerning these other factors adds uncertainty to any interpretation. Habituation and tolerance to drugs and to the effects of drugs develop with chronic use and are highly variable among different individuals, further complicating interpretations, especially those based on individual drug-use testing results. Therapeutic drugs, even if medically indicated and prescribed, can have ambiguous effects with respect to on-the-job safety or impairment of individuals. Some are psychoactive and can potentially cause impairment for a given task or responsibility or have other adverse effects. However, as pointed out by a consensus panel on drug concentrations and driving impairment, in certain persons and conditions the adverse effects of psychoactive drugs in therapeutically effective concentrations are deemed to pose much less risk than the disorders they treat. The examples cited were
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chronically anxious, preoccupied, and irritable persons treated with anxiolytic drugs; schizophrenic or depressed persons treated appropriately with antipsychotic or antidepressant medications; and persons with epilepsy treated with anticonvulsant drugs. These considerations apply equally to workplaces and environments other than a motor vehicle.

The foregoing generalizations apply to the interpretation of qualitative and quantitative results in any biological specimen. Additional specific limitations on the significance of drug-use testing results apply to tests on hair or on urine. Hair serves as a depository for certain drugs and toxic substances (e.g., heavy metals). Its analysis, however valid and sophisticated, can only at best provide a retrospective historical record of exposure to drugs. Such an analysis can provide some indication of when and in what kind of pattern the person used or was exposed to the drugs. Negative test results can represent threshold situations and do not conclusively eliminate prior drug intake or exposure. Positive results do not lend themselves to meaningful estimates of the drug doses taken or intensity of exposure, at least not with current available information. At present, the most useful application of drug analyses in hair might be to resolve a question of recent one-time use of or exposure to drugs, as differentiated from intermittent or continuous chronic use over a prolonged past period of time.

Urine is widely tested for drugs despite its many limitations discussed above and summarized in Table 2. The lack of close correlation between blood and urine alcohol concentrations and between urine-alcohol concentrations and driving impairment has been so thoroughly established and recognized that only 33 of the 50 states now allow urine to be analyzed for alcohol in connection with the investigation of alcohol-related traffic offenses. Impairment of the ability safely to carry out any given task or responsibility cannot be established or even presumed from the results of drug tests in urine, whether qualitative or quantitative. Neither can absence of drug-related impairment be established from the results of drug tests in urine, regardless of the analysis procedures used. Nor is there a consistent and predictable relationship between the quantity (dose) and time of administration or exposure to drugs and the resultant drug or drug metabolite concentrations in urine, especially in single random urine specimens. Moreover, many drug metabolites found in urine are pharmacologically inactive, and the

active present drug species are either not present in urine or only occur in concentrations too low to be readily and reliably measured. This is the usual situation after use of marihuana or cocaine, for both of which the primary urinary metabolites are pharmacologically inactive. The widely variable period of urinary excretion of such metabolites after last use of a drug adds a further uncertain element and constraint on meaningful interpretation of positive or negative urine drug test results for other purposes than simply to document presence or absence of drugs of interest or their metabolites in the particular urine specimen analyzed. The significance of drug determinations in urine was well summarized by the 1983 Consensus Development Panel on Drug Concentrations and Driving Impairment. Its statement on that subject, quoted in its entirety, follows:

Testing of drugs or drug metabolites in urine is only of qualitative value in indicating some prior exposure to specific drugs. Inferences regarding the presence or systemic concentration of the drug at the time of driving or impairment from drug use are generally unwarranted. The presence of an illicit substance in urine that may indicate prior illegal action can, however, add a dimension to probable cause of observed driving performance. 182

That statement is equally applicable to the issue of drug-related impairment for tasks other than driving. Documentation of the tenuous nature of the relationship between urine drug concentrations and impairment or other effects on the subject is afforded by the outcome of a recent comprehensive evaluation of existing data on concentrations of cannabinoids, diazepam, diphenhydramine, methaqualone, and secobarbital in blood, saliva, and urine with respect to their applicability to the detection of drug-impaired driving. 183 The study used pharmacokinetic methods for the attempted correlation of urine and saliva drug concentrations to those in blood which were presumably related to presence of behavioral impairment in laboratory tasks. The study concluded that

marihuana was the only drug for which sufficient data were available to suggest the use of urine tests to establish the need to obtain

181. See Blanke, supra note 7.
182. Id.
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181. See Blanke, supra note 7.

182. Id.

or analyze a blood specimen for THC. Data from numerous studies support the proposal that testing for THC metabolites in urine or at above the 100 ng/ml concentration will provide better than a 50% probability of detecting levels of THC in the blood that may be associated with impairment. . . . At the present state of knowledge, blood is the only body fluid that may serve in a limited manner to relate drug levels to impaired driving.184

A 50% probability, of course, indicates identical likelihood that the contemplated event (cannabinoids concentration in blood associated with impairment) will occur or not occur; a coin flip or yes-no guess would have that same probability of producing the correct answer. The study report does not address how much better than 50% the probability for the associated events is, but simply states that “this preliminary assessment suggested that the measurement of THC-9-acid concentrations in urine might provide a better than even chance to predict levels of THC that could be associated with impairment.”185

There is also the question of the prolonged effects or consequences of alcohol and other drug use after discontinuation of acute drug intake. Hangover and withdrawal syndromes and carry-over effects can be present and affect a subject even if no drugs are detectable in the urine (or other biofluids). One recent study of pilot performance on flight-simulator landing tasks showed measurably impaired performance in these tasks up to 24 hours after 10 subjects had each smoked one marihuana cigarette containing 19 mg of ∆9-THC, although the pilots reported no significant subjective awareness of impaired performance at 24 hours.186 A comparable study of 10 pilots under simulated flying conditions after use of alcohol instead of cannabis showed continued impairment 14 hours after alcohol consumption and after the alcohol had disappeared from the blood, despite general failure of the pilots to note subjective hangover effects.187 Clearly, absence of detectable drugs in urine (or other biological fluids) and lack of subjective perceptions of impairment do not per se establish the absence of drug-induced impairment or of the after-effects of drug use.

Separate and somewhat special considerations apply to the use of

184. Id.
185. Id.

C. Relationship of Drug Dose, Route of Administration, Time and Concentration

This subheading is, in fact, a rough approximation of the subject matter encompassed in the field of pharmacokinetics. Clearly, a detailed consideration of these matters is beyond the scope of this article, but some generalizations seem pertinent and useful. The sojourn of a drug in the human body can be depicted as a sequence of events: Absorption — distribution — biotransformation — translocation — excretion. A much simplified scheme for this process is illustrated in Figure 1.

188. Gold & Dackis, Role of the Laboratory in the Evaluation of Suspected Drug Abuse, 47 J. CLIN. PSYCHIATRY 17 (1986); Kamerow, Pincus, & MacDonald, Alcohol Abuse, Other Drug Abuse, and Mental Disorders in Medical Practice: Prevalence, Costs, Recognition, and Treatment, 255 J. A.M.A. 2054 (1986).
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**Absorption:** The drug, whether ingested, injected or absorbed through mucous membranes or other portals becomes freely absorbable at the site of administration. It reaches the systemic circulation either directly by injection, or indirectly by diffusion or active transport through cell membranes. The bioavailability of the drug partly controls the proportion of an administered dose which eventually reaches the systemic circulation; injection and inhalation are more efficient in this respect than oral intake.

**Distribution:** The free drug which has reached the systemic blood can remain dissolved in the plasma water, and/or be shifted into blood cells, or bound reversibly to plasma proteins. The systemic circulation carries the free or bound drug to all tissues and organs of the body, where the free drug can exert effects, or be stored, excreted, and metabolized or otherwise biotransformed, depending upon the organ, tissue and drug. Some tissues and organs merely act as passive, unaffected reservoirs, thus influencing the equilibrium concentration of the drug in plasma. Other organs, such as the lungs and especially the liver, convert the parent drug into chemically different metabolites or biotransformation products, in which forms it can be stored, or eliminated by excretion in feces, urine, saliva, and sweat or reabsorbed from the bile and other sites into the systemic circulation for distribution to all tissues and organs. The metabolites can be pharmacologically inert, or possess activity which is less than, equal to, or greater than the parent drug. This interactive process can continue, with production of additional biotransformation products, until all of the drug has been eliminated from the body. All of the foregoing processes are time dependent, with rates — sometimes interrelated — which control the respective concentrations of free and bound drug in the plasma and tissues at any given time after administration of a given drug dose.

**Effects:** Taken together, the foregoing processes and the excretion and elimination of the drug produce a pattern of concentration versus time course which can be represented by simple linear or by very complex nonlinear mathematical models. These features control, in large measure, the quantity of a drug which reaches accessible tissue sites of drug action in target organs such as the brain. There, in competition with other drugs or biochemical compounds, the drug binds to or combines reversibly or nonreversibly (depending on the drug) to general or specific receptors. This process, in essence, causes the particular pharmacological actions and effects of the drug which are part of its inherent characteristics, doing so in concentration- and rate-related fashion which governs the speed of onset, duration, and intensity of the drug effects.

**Elimination and Excretion:** Drug disposition occurs primarily through metabolism or biotransformation into active or inert substances that can either take part in other biochemical events in the body in their altered form, or be excreted; and through excretion of the parent drug through such organs as the kidneys, intestines, lungs, and skin. It follows that unaltered parent drugs can be excreted in urine, feces, breath, and sweat depending upon the drug. Some drugs, e.g., ethanol, are predominantly disposed of through extensive metabolism in the liver, while others are not completely metabolized and are chiefly excreted in body waste products. In general, metabolites are more polar substances than the parent drug, thus facilitating their excretion through aqueous channels as in urine, in distinction to the less polar parent drugs which tend to be lipophilic and thus preferentially parti-
FIGURE 1. Simplified Scheme of the Interrelationships of Drug Absorption, Distribution, Biotransformation, and Excretion. Modified from Benet & Sheiner. 189

Absorption: The drug, whether ingested, injected or absorbed through mucous membranes or other portals becomes freely absorbable at the site of administration. It reaches the systemic circulation either directly by injection, or indirectly by diffusion or active transport through cell membranes. The bioavailability of the drug partly controls the proportion of an administered dose which eventually reaches the systemic circulation; injection and inhalation are more efficient in this respect than oral intake.

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tioned into adipose (fatty) tissues in the brain and elsewhere. The renal excretion process for drugs and their metabolites involves filtration, secretion, and reabsorption in various combinations and at different rates for different drugs. These processes are affected by the bound/free drug proportions, polarity of metabolites, the pH and flow rate of urine and many other factors. Only unbound drugs and metabolites are filtered by the undamaged kidneys and, in general, are concentrated in the urine leaving the kidneys. These several factors and processes, together with other important factors such as the drug concentration in the plasma, affect the ultimate concentration of drugs and metabolites in ureteral urine. The bladder urine concentrations of drugs and metabolites, and hence those of the voided urine, are further affected by accumulation of drug-free urine before and after the period of urinary drug/metabolite excretion. There are also frequent and wide fluctuations in the concentration of some drugs, such as amphetamines and cocaine, in urine or saliva, resulting from pH changes and other factors. These events and biological and chemical factors combine to make urine concentrations of drugs and metabolites as a function of time a highly variable phenomenon, which does not necessarily closely reflect the body burden of a given drug, or its dose.

Combinations and permutations of these processes and the underlying drug intake patterns can, therefore, result in absence, or presence at varying concentrations, of drugs in urine and in other biological fluids after supposedly identical drug intake in different persons at the same time, and in the same person on different occasions. Many of these matters are considered and discussed in detail in a recent monograph on the pharmacokinetics and pharmacodynamics (mode of action) of psychoactive drugs.190

D. Limitations of Drug-Use Testing of Urine

Although urine as a specimen for drug tests and the nature and probative value of drug-use testing results on urine have been discussed in relation to various topics already considered in this article, it seems useful to summarize what can and cannot be learned from drug testing in urine. That subject is widely misunderstood and the facts are occasionally misrepresented by parties with special interests or points of view. With few, if any, exceptions, testing for drugs in urine presents no unique technical difficulties. In many instances, drugs or metabolite concentrations in urine are, in fact, higher than those of the same drug in other accessible biological fluids, such as plasma or saliva.

Urine is subject to biological and chemical change and decomposition at room temperature and even under refrigeration at 4°C unless chemically treated; and those changes can affect the result of some tests. However, analysts and laboratories generally prefer that no chemical preservatives be added to urine, because such additives can also add an uncertainty element to test performance and test results, especially to testing by immunochemical methods. At most, steps should be taken to keep the urine at or below a pH of 7.0, so as to prevent ammonial decomposition. When feasible, keeping urine frozen at -20°C or lower temperatures will minimize or altogether prevent changes in chemical composition. Storage in the frozen state is recommended practice for the remaining portions of tested urines which have yielded results positive for presence of drugs, with precautions to prevent container rupture through expansion of specimens as they reach the frozen state.

The absence or presence of target drugs or drug metabolites in urine can be established with adequate validity, using reasonable pre-established cutoff values or threshold concentrations appropriate for each target drug or metabolite as a major criterion for designating the test results as negative or positive. Individual positive results, even when properly confirmed in analytical terms, and with rare exceptions, cannot be used to establish what dose of the drug in issue was taken, or when it was taken, or the time lapse since last intake of or exposure to the drug, nor to determine whether the drug was taken chronically and, if so, for how long prior to the collection of the tested urine specimen. For a variety of drugs, it has been demonstrated that "passive" exposure, e.g., by inhalation of ambient air by a nonsmoking person in the vicinity of heavy marijuana smokers in a confined environment or small space, can cause positive drug test results in urine which cannot be readily differentiated from those produced by active use of the drug. The same situation holds for inability to differentiate positive results in urine for cannabinoids, cocaine, or morphine yielded by ingestion of foods or drink containing those drugs from those results yielded by active, intentional taking of low doses of those drugs in conventional drug form. It is very likely that further studies will reveal other instances of "innocent" or unknowing consumption of drugs in food or drink which lead to positive drug-use testing results in urine mimicking low dose deliberate drug intake, like those documented for morphine from opium.

tioned into adipose (fatty) tissues in the brain and elsewhere. The renal excretion process for drugs and their metabolites involves filtration, secretion, and reabsorption in various combinations and at different rates for different drugs. These processes are affected by the bound and free drug proportions, polarity of metabolites, the pH and flow rate of urine and many other factors. Only unbound drugs and metabolites are filtered by the undamaged kidneys and, in general, are concentrated in the urine leaving the kidneys. These several factors and processes, together with other important factors such as the drug concentration in the plasma, affect the ultimate concentration of drugs and metabolites in ureteral urine. The bladder urine concentrations of drugs and metabolites, and hence those of the voided urine, are further affected by accumulation of drug-free urine before and after the period of urinary drug/metabolite excretion. There are also frequent and wide fluctuations in the concentration of some drugs, such as amphetamines and cocaine, in urine or saliva, resulting from pH changes and other factors. These events and biological and chemical factors combine to make urine concentrations of drugs and metabolites as a function of time a highly variable phenomenon, which does not necessarily closely reflect the body burden of a given drug, or its dose.

Combinations and permutations of these processes and the underlying drug intake patterns can, therefore, result in absence, or present at varying concentrations, of drugs in urine and in other biological fluids after supposedly identical drug intake in different persons at the same time, and in the same person on different occasions. Many of these matters are considered and discussed in detail in a recent monograph on the pharmacokinetics and pharmacodynamics (mode of action) of psychoactive drugs. 190

D. Limitations of Drug-Use Testing of Urine

Although urine as a specimen for drug tests and the nature and probable value of drug-use testing results on urine have been discussed in relation to various topics already considered in this article, it seems useful to summarize what can and cannot be learned from drug testing in urine. That subject is widely misunderstood and the facts are occasionally misrepresented by parties with special interests or points of view. With few, if any, exceptions, testing for drugs in urine presents no unique technical difficulties. In many instances, drugs or metabolite concentrations in urine are, in fact, higher than those of the same drug in other accessible biological fluids, such as plasma or saliva.

Urine is subject to biological and chemical change and decomposition at room temperature and even under refrigeration at 4°C unless chemically treated; and those changes can affect the result of some tests. However, analyses and laboratories generally prefer that no chemical preservatives be added to urine, because such additives can also add an uncertainty element to test performance and test results, especially to testing by immunochemical methods. At most, steps should be taken to keep the urine at or below a pH of 7.0, so as to prevent ammoniacal decomposition. When feasible, keeping urine frozen at -20°C or lower temperatures will minimize or altogether prevent changes in chemical composition. Storage in the frozen state is recommended practice for the remaining portions of tested urines which have yielded results positive for presence of drugs, with precautions to prevent container rupture through expansion of specimens as they reach the frozen state.

The absence or presence of target drugs or drug metabolites in urine can be established with adequate validity, using reasonable pre-established cutoff values or threshold concentrations appropriate for each target drug or metabolite as a major criterion for designating the test results as negative or positive. Individual positive results, even when properly confirmed in analytical terms, and with rare exceptions, cannot be used to establish what dose of the drug in issue was taken, or when it was taken, or the time lapse since last intake of or exposure to the drug, nor to determine whether the drug was taken chronically and, if so, for how long prior to the collection of the tested urine specimen. For a variety of drugs, it has been demonstrated that “passive” exposure, e.g., by inhalation of ambient air by a nonsmoking person in the vicinity of heavy marihuana smokers in a confined environment or small space, can cause positive drug test results in urine which cannot be readily differentiated from those produced by active use of the drug.

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poppy seeds in baked goods and other foods.

Some drugs and or drug metabolites are prone to long-term storage in body tissues, for weeks or months. As exemplified by the cannabinoids from marihuana, drugs so stored can continue to be excreted for days, weeks, and even months after the last drug intake. Because of the concentrating effect of the usual renal excretion process for drugs, some such drugs can reach readily detectable concentrations for a prolonged period after assured abstinence. Hence, presence of detectable concentrations of cannabinoids, and some other drugs, in single or even in serial urine specimens does not per se equate with recent use of the drug.

Lastly, drug-induced intoxication, impairment, or other effects on a person at any given time cannot be established or even validly presumed from a urine test result, or a series of such results. These limitations arise from the nature of urinary drug excretion, which is often in the form of pharmacologically inactive drug metabolites, from the well-documented absence of correspondence between drug effects on the person and urine concentrations of psychoactive drugs, and from the low correlation of these variables.

E. Typical Periods of Drug Detectability After Use

Many of the factors affecting the length of time, after use or last use, during which a drug is detectable in biological tissues and fluids have been discussed earlier in this article. Because urine is at present the most frequently and most commonly selected specimen for drug use testing, this consideration of the length of time for which a drug remains detectable will be limited to its presence in urine. The ultimate controlling variables are the nature and identity of the drug and/or metabolites and the concentration of those analytes in the urine specimen; and those two variables are the outcome of all other biological, chemical and pharmacokinetic factors and events.

For simplicity and brevity, the information on the duration of drug detectability after last use is presented in tabular form. Commonly used and typical operational limits of sensitivity and cutoff concentrations are listed. It is understood that higher sensitivities are attainable for many drugs under special conditions, and that the lower detection limits could extend the time period of drug detectability after last use. The data appearing in Table 9 are approximate and are subject to change or modification in the light of additional and newer information which is certain to accrue from further studies and additional experience. Those data are excerpted from references cited in this article and other sources believed to be authoritative; but individual references have been omitted for reasons of expediency. A widely used reference on the disposition of toxic drugs and chemicals in man was published in 1982 and includes some literature citations through 1981. Finally and once more, the data in Table 9 relate only to the detectability of drugs as a function of time by typically available drug-use testing procedures; they have no relation to existence or manifestations of impairment or other drug effects.

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191. In the context of this article, the author considers that intoxication is inability or unfitness safely to perform the task in issue, as the result of the acute effects of drugs upon the central nervous system and/or other body organs and functions; and that impairment is a decrease in fitness safely to perform the task in issue, as the result of the acute effects of drugs on the central nervous system or other body organs and functions.

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<td></td>
<td>1 - 3</td>
</tr>
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<td></td>
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</tr>
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</tr>
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<td>2 - 21</td>
</tr>
<tr>
<td>Cocaine metabolites</td>
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<td>2 - 5</td>
</tr>
<tr>
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<td>100 (mcg/ml)</td>
<td>1/2 - 1</td>
</tr>
<tr>
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<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Methadone</td>
<td>300</td>
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</tr>
<tr>
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<td>300 - 1000</td>
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</tr>
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VII. Regulation of Drug-Use Testing

For an activity which has affected millions of persons in a most direct way and will obviously continue to affect many more millions, especially those in the active private work force or in governmental employ, drug-use testing has been almost uniquely unregulated or under-regulated. A Federal licensure scheme for clinical laboratories and clinical laboratory personnel, performing functions and tests very comparable to drug-use testing, has been in operation for twenty years, since passage of the Clinical Laboratories Improvement Act of 1967. The Congress mandated in that act that the Secretary of Health and Human Services promulgate standards for clinical laboratories operating directly or indirectly in interstate commerce, and provided that such standards shall be designed to assure consistent performance by the laboratories of accurate laboratory procedures and services, and shall include, among others, standards to assure:

(i) Maintenance of a quality control program adequate and appropriate for accuracy of the laboratory procedures and services;
(ii) Maintenance of records, equipment and facilities necessary to proper and effective operation of the laboratory;
(iii) Qualifications of the director of the laboratory and other supervisory professional personnel necessary for adequate and effective supervision of the operation of the laboratory (which shall include criteria relating to the extent to which training and experience shall be substituted for education); and
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Substantial improvements in clinical laboratory test performance have been documented, by proficiency testing and/or other means, since these nationwide standards became effective. Substantially the same standards and comparable implementing regulations have been mandated by the federal government for hospital and independent clinical laboratories as a condition of their eligibility for coverage of services under Medicare and other elements of the Social Security Act. Thus, it has been recognized that the public interest requires regulation of facilities and personnel engaged in the production of information by “examination of materials derived from the human body.” Further, the principles and practice of such governmental regulation at the Federal, state, and local level are long established and widely understood. To be sure, these existing regulatory schemes were imposed only on “clinical laboratories” which are facilities carrying out examinations of human biological specimens to provide information “for the diagnosis, prevention, or treatment of any disease or impairment of, or the assessment of the health of, man.” 195 There is, however, no reason to omit comparable regulation of drug-use testing laboratories and activities.

A. Lack of Universal Accreditation or Licensure Systems

The vagaries and limitations of the governmental regulatory schemes applicable to clinical laboratories have resulted in excluding from that regulation those facilities engaged only in drug-use testing for nonmedical purposes and the nonmedical drug-use testing activities of clinical laboratories which are regulated in other respects. Hence, there is no nationwide licensure system in place at the federal level to control and regulate drug-use testing establishments, their personnel, activities, operations, or performance. Neither have the several states so far enacted licensure or accreditation laws for the persons and establishments engaged in nonmedical drug-use testing. It is clear that drug-use testing for medical purposes is encompassed under the general federal and state regulatory umbrellas for biological, chemical, biophysical or other examinations on human biological specimens for diagnostic and therapeutic or other medical purposes. These medical applications of drug-use testing include emergency toxicology for drug overdose diagnosis and treatment, and the diagnosis and treatment of drug addiction and dependency and of other drug-related ailments and infirmities which are medically and legally recognized. Clearly not covered by existing clinical laboratory licensure laws is drug-testing carried out for exclusively nonmedical, forensic applications such as tests for alcohol and other intoxicating substances for traffic law enforcement. That situation has been well settled by administrative and judicial decisions and by statutory enactments which preempt regulation of such forensic drug-testing activities, but which apply only to the narrow purposes stated in those statutes, predominantly land, water and air traffic law enforcement.

The drug-use testing field is not, however, entirely unregulated. Specific occupational and some nonoccupational activities are the basis for regulated drug-use testing under federal laws. That regulation does not, however, as a rule include licensure or accreditation of laboratories or laboratory personnel. Examples of activities covered by such federally regulated testing are railroad operations, commercial and noncommercial aviation, the military services which have their own extensive set of Department of Defense-wide policies and procedures, and, of course, drug-use testing of Federal employees in sensitive positions and under other defined circumstances under authority of Executive Order 12,564 issued by the President of the United States on September 15, 1986. 196 There is also the extensive set of private-sector policies for drug-use testing in connection with intercollegiate athletics under the jurisdiction of the National Collegiate Athletic Association (NCAA). 197 Those policies deal with such details as listing of 75 prescribed drugs including anabolic steroids, and specimen collection procedures; but they do not address the qualifications of the testing laboratories or laboratory personnel. 198 The NCAA list of “banned” drugs is a prime example of unselective overkill. It includes anachronisms such

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\textsuperscript{198} Id.
as the listing of strychnine, and misspellings, but it omits altogether hallucinogens such as LSD and PCP as well as opiates, sedatives, and analgesics. Further, the gratuitous addition of "AND RELATED COMPOUNDS" to each drug category except "Street Drugs" where the term "OTHERS" is added, renders the prohibitions so vague and indefinite as to prevent any person affected by these rules from reasonably concluding whether any given drug or medicament would fall within the intended prohibitions. Because the NCAA "Banned Drug List 1986" is new, extensive and unusual, it is included herein as Table 10, verbatim, for ready reference.

Table 10. NCAA Banned Drugs List 1986***
(With Examples)

A. Psychomotor stimulants:
- amphetamine
- benzphetamine
- chlorphentetamine
- cocaine
diethylpropion
dimethylamphetamine
ethylamphetamine
fencamfamine
meclofenoxate
methylamphetamine

B. Sympathomimetic amines:
- chlorpromazine
- ephedrine
- etadrenaline
- isometheamine
- isoproterenol

C. Miscellaneous central nervous system stimulants:
- amphetanole
- benzylidene
- caffeine
- cipropramide
crotethamide
doxapram

D. Anabolic Steroids:
- clotebol
dehydrobromotestosterone
- fluoxymesterone
- mesterolone
- methenolone
- methandienone
- nandrolone

E. Substances banned for specific sports:
Rifle:
- alcohol
- atenolol
- metoprolol
- nadolol

G. Street Drugs:
- methamphetamine
- cocaine
- brom
- marijuana

199. Id.

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D. Anabolic Steroids:
- clotebol
dehydrobromotestosterone
- fluoxymesterone
- mesterolone
- methenolone
- methandienone
- nandrolone

E. Substances banned for specific sports:
Rifle:
- alcohol
- atenolol
- metoprolol
- nadolol

F. Diuretics:
- bendroflumethiazide
- benzthiazide
- bumetanide
- chlorothiazide
- chlorothalidone
cylothiazide
- ethacrynic acid
- furosemide
- hydrochlorothiazide

G. Street Drugs:
- methamphetamine
- cocaine
- brom
- marijuana

Definition of positive depends on the following:

*For caffeine — if the concentration in urine exceeds 15 micrograms/mL.
*For testosterone — if the ratio of the total concentration of testosterone to that of epistemosterone in the urine exceeds it.
*For marijuana and THC — based on a repeat testing.

H. Substances Given Special Consideration:
[Note: Usage of these substances may or may not be permissible, depending on limitations expressed in the following guidelines and/or quantities used of these substances.]

1. Blood Doping. The practice of blood doping (the intravenous injection of whole blood, packed red blood cells or blood substitutes), as well as the use of growth hormone (human, animal or synthetic), is prohibited and any evidence confirming use may be cause for punitive action.

2. Local Anesthetics. The NCAA Executive Committee will not be opposed to the limited use of local anesthetics under the following conditions:
(a) That procaine, xylcaine, carbocaine without epinephrine, or any other vaso-constrictor may be used, but not cocaine;
(b) That only local or topical injections can be used (i.e., intravenous injections
as the listing of strychnine, and misspellings, but it omits altogether hallucinogens such as LSD and PCP as well as opiates, sedatives, and analgesics. Further, the gratuitous addition of “AND RELATED COMPOUNDS” to each drug category except “Street Drugs” when the term “OTHERS” is added, renders the prohibitions so vague as to be indefinite as to prevent any person affected by these rules from reasonably concluding whether any given drug or medicament would fall within the intended prohibitions. Because the NCAA “Banned Drug List 1986” is new, extensive and unusual, it is included herein as Table 10, verbatim, for ready reference.

Table 10. NCAA Banned Drugs List 1986
(With Examples)

A. Psychomotor stimulants:

- amphetamine
- benzphetamine
- chlorphenthimine
- cocaine
- diethylpropion
- dimethylamphetamine
- ethylamphetamine
- fenfluramine
- methcathexate
- methylamphetamine

B. Sympathomimetic amines:

- chlorphenamine
- ephedrine
- etadrenaline
- isoprenaline
- isoprenaline

C. Miscellaneous central nervous system stimulants:

- amiphenazole
- benzphetamine
- caffeine
- cefopropamide
- cefotiamide
- doxapram

D. Anabolic Steroids:

- cloestebol
- dehydrochlormethyl-testosterone
- fluoxymesterone
- mesterolone
- methandrostenolone
- nandrolone

E. Substances banned for specific sports:

- alcohol
- atenolol
- metoprolol
- nadolol

F. Diuretics:

- bendroflumethiazide
- benhaizide
- bumetanide
- chlorthiazide
- chlorthalidone
- cyclothiazide
- ethacrynic acid
- flumethiazide
- furosemide
- hydrochlorothiazide

G. Street Drugs:

- amphetamine
- cocaine
- heroin
- marijuana

Definition of positive depends on the following:

*For caffeine — if the concentration in urine exceeds 15 micrograms/ml.
*For testosterone — if the ratio of the total concentration of testosterone to that of epitestosterone in the urine exceeds it.
*For marijuana and THC — based on a repeat testing.

H. Substances Given Special Consideration:

[Note: Usage of these substances may or may not be permissible, depending on limitations expressed in the following guidelines and for quantities used of these substances.]

1. Blood Doping. The practice of blood doping (the intravenous injection of whole blood, packed red blood cells or blood substitutes), as well as the use of growth hormone (human, animal or synthetic), is prohibited and any evidence confirming use may be cause for punitive action.

2. Local Anesthetics. The NCAA Executive Committee will not be opposed to the limited use of local anesthetics under the following conditions:

(a) That procaine, xylocaine, carbocaine without epinephrine, or any other vaso-constrictor may be used, but not cocaine;

(b) That only local or topical injections can be used (i.e., intravenous injections

https://nsuworks.nova.edu/nlr/vol11/iss2/22
are not permitted);

(c) That use is medically justified only when permitting the athlete to continue the competition without potential risk to his health.

The NCAA crew chief in charge of testing must be advised in writing by the team physician if the anesthetic has been administered within 24 hours of the competition. He must also be advised of time, route and dose of administration.

3. Asthma or Exercise-Induced Bronchospasm.

The use of three beta-agonists, Terbutaline, Salbutamol and Bitolterol, for the treatment of asthma are approved under the following condition: The treating doctor must notify the crew chief beforehand that such a patient has asthma and that he is using, or may require the use of, one or all of the drugs. Requests must be in writing identifying the drugs, dose and frequency of administration. All other sympathomimetic amines are banned. Drugs related to Cromolyn Sodium, Aminophylline and Theophyllines, Beclomethasone and Dipotassium Sulfate may be used.

4. Corticosteroids. The NCAA has become increasingly concerned by the misuse of corticosteroids in some sports.

The Executive Committee therefore has decided that the use of these drugs at NCAA championships or certified football bowl games must be declared. A doctor using them must state in writing to the crew chief the name of the competitor being treated; the name, dose and route of administration of the drug; the reason for this use; the date of administration; the time of administration and the name and signature of the doctor.

It seems likely that the series of recent federal government policies and regulations concerning drug-use testing of certain federal employees under defined conditions will ultimately result in more pervasive regulation in at least three ways: 1) The federal regulations themselves are certain to be extended to currently unregulated aspects of drug-use testing such as accreditation or licensure of laboratories; 2) the states and some localities will enact laws and ordinances modeled after the federal regulations or key aspects thereof; and 3) marketplace competition, litigation and arbitration, and other external forces will combine to shape the practices of drug-use testers into a more rigid conformity to those mandated by law for other tested populations such as the federal workforce. The federal initiatives so far involve four separate elements: 1) A Presidential Executive Order; 2) Office of Personnel Management Guidelines for testing and counseling programs of federal agencies; 3) Department of Health and Human Services Scientific and Technical Guidelines for Drug Testing Programs; and 4) Draft Standards for Accreditation of Laboratories Engaged in Urine Drug Testing, issued by the National Institute on Drug Abuse.

The President’s Executive Order on the “Drug-Free Federal Workplace” among other directives mandated that each executive agency establish a program to test for the use of illegal drugs by employees in “sensitive positions” and a program for voluntary employee drug-testing. It also authorizes drug-use testing of executive agency employees when there is reasonable suspicion that any employee uses illegal drugs, or in an examination authorized by the agency regarding an accident or unsafe practice, or as part of or as a follow-up to counseling or rehabilitation for illegal drug use through an Employee Assistance Program; and it allows testing of any applicant. The Office of Personnel Management was mandated to issue Government-wide guidance on the implementation of the terms of the Executive Order. Executive agencies were mandated to conduct their drug-testing programs in accordance with scientific and technical guidelines to be promulgated by the Secretary of Health and Human Services. A specific mandate is that “procedures for providing urine specimens must allow individual privacy, unless the agency has reason to believe that a particular individual may alter or substitute the specimen to be provided.” Alcohol misuse or abuse is not addressed in this Executive Order. Executive Order 12,564 became effective upon proclamation on 15 September 1986.

The Office of Personnel Management (OPM) on November 28, 1986 issued its “Government-wide Guidance” to executive agencies as FPM letter 792. Among other provisions, the OPM guidance document provides, with regard to applicant testing, that “Agencies should include notice of drug testing on vacancy announcements for those positions where drug testing is required.” The OPM guidance also provides that notices concerning drug-use testing be given to employees in a “testing designated position” and that they include “[a]ssurance that the quality of testing procedures is tightly controlled, that the test used to confirm use of illegal drugs is highly reliable, and that test results
are not permitted);

(c) That use is medically justified only when permitting the athlete to compete without potential risk to his health.

The NCAA crew chief in charge of testing must be advised in writing by the toxicologist if the anesthetic has been administered within 24 hours of the competition. He must also be advised of time, route and dose of administration.

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4. Corticosteroids. The NCAA has become increasingly concerned by the misuse of corticosteroids in some sports.

The Executive Committee therefore has decided that the use of these drugs in NCAA championships or certified football bowl games must be defined: a doctor using them must state in writing to the crew chief the name of the competitor being treated; the name, dose and route of administration of the drug for this use; the date of administration; the time of administration and the name and signature of the doctor.

It seems likely that the series of recent federal government policy and regulations concerning drug-use testing of certain federal employees under defined conditions will ultimately result in more precise regulation in at least three ways: 1) The federal regulations themselves are certain to be extended to currently unregulated aspects of drug-use testing such as accreditation or licensure of laboratories; 2) the states and some localities will enact laws and ordinances modeled after the federal regulations or key aspects thereof; and 3) marketplace competition, litigation and arbitration, and other external forces will combine to shape the practices of drug-use testers into rough conformity to those mandated by law for other tested populations such as the federal workforce. The federal initiatives so far involve four separate elements: 1) A Presidential Executive Order; 2) Office of Personnel Management Guidelines for testing and counseling programs of federal agencies; 3) Department of Health and Human Services Scientific and Technical Guidelines for Drug Testing Programs; and 4) Draft Standards for Accreditation of Laboratories Engaged in Urine Drug Testing, issued by the National Institute on Drug Abuse.

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200. See Executive Order supra note 196.
201. The term “illegal drugs,” as defined in the Executive Order, means a controlled substance included in Schedule I or II of the Controlled Substances Act, as defined in Section 802(6) of Title 21 of the United States Code, the possession of which is unlawful under Chapter 13 of that Title. The term “illegal drugs” does not mean the use of a controlled substance pursuant to a valid prescription or other uses authorized by law. See Executive Order supra note 196.
202. Id.
204. Id.
will be handled with maximum respect for individual confidentiality, consistent with safety and security. 868 The OPM guidance defines "reasonable suspicion" of drug use as "an articulable belief that an employee uses illegal drugs drawn from specific and particularized facts and reasonable inferences from those facts" and exemplifies the latter as follows:869

- Observable phenomena, such as direct observation of drug use and/or the physical symptoms of being under the influence of a drug
- A pattern of abnormal conduct or erratic behavior
- Arrest or conviction for a drug related offense; or the identification of an employee as the focus of a criminal investigation into illegal drug possession, use, or trafficking
- Information provided either by reliable and credible sources or independently corroborated
- Newly discovered evidence that the employee has tampered with a previous drug test.

The initial HHS guidelines entitled "SCIENTIFIC AND TECHNICAL GUIDELINES FOR DRUG TESTING PROGRAMS" were prepared by the Alcohol, Drug Abuse, and Mental Health Administration (ADAMHA) of the Department of Health and Human Services and are dated February 13, 1987.870 They are too lengthy and detailed to be fully summarized in this article, but several aspects of the four Sections of Part I of the document (The Drugs, Specimen Collection Procedures, Laboratory Analysis Procedures, and Reporting and Review of Results) are worth noting briefly. Executive agency drug-use testing programs must test for marihuana and cocaine. They may also test routinely for amphetamines, opiates and phencyclidine, and may also test for any drug listed in Schedule I or II of the Controlled Substances Act when conducting reasonable suspicion testing. Urine collection procedures are detailed. They provide, that a minimum of 60 ml of urine is to be collected, that the time from urination to delivery of the sample should in no case exceed four minutes, that the temperature of the urine specimen is to be measured immediately and if outside the range of 32.5 - 37.7°C will give rise to reasonable suspicion of adulteration/substitution, and that toilet bluing agents are to be placed in toilet tanks and urinals as a precaution against unrecognized dilution of a urine specimen. Urine specimens are to be refrigerated upon arrival at the laboratory if not initially tested within two days. All specimens yielding confirmed positive test results must be retained in properly secured freezer storage for at least 365 days. Initial testing to eliminate drug-negative specimens is to be conducted with an immunoassay method meeting the requirements of the Food and Drug Administration for commercial distribution, or by thin-layer, high pressure liquid or gas chromatography if immunoassays are unavailable for specific drugs of concern. All specimens yielding positive initial test results must be subjected to confirmatory testing using quantitative gas chromatography/mass spectrometry. The following initial and confirmatory cutoff concentrations are mandated for determining whether the test results are positive or negative:

<table>
<thead>
<tr>
<th>Drug</th>
<th>Initial Test Cutoff Concentration, ng/ml</th>
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<tbody>
<tr>
<td>Amphetamines</td>
<td>1000</td>
<td>300</td>
</tr>
<tr>
<td>Cocaine Metabolites (Benzoylcegonine)</td>
<td>300</td>
<td>150</td>
</tr>
<tr>
<td>Cannabinoids</td>
<td>100</td>
<td>20</td>
</tr>
<tr>
<td>Δ⁹-THC-9-carboxylic acid</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>Opiates</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Phencyclidine</td>
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<td></td>
</tr>
</tbody>
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Confirmation of drugs is to be by means of an accepted quantitative GC/MS procedure; if none exists for a given drug of interest, preference will be given to confirmation by a full-scan GC/MS analysis and quantitation by an alternate chromatographic method. All methods must meet "commonly accepted analytical standards." Only specimens with positive results upon confirmation are to be reported as positive for a given drug. Verbal telephone reports are not permitted. All records pertaining to a given urine specimen must be retained by the laboratory for at least two years. In regard to the restesting of specimens, it is stated that "some analytes deteriorate or are lost during freezing and/
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or storage." All laboratories must have the capability and facilities for performing both screening and confirmatory tests for each drug or metabolite for which service is offered. Comprehensive quality assurance and quality control procedures are mandated. These must include analysis of QC specimens blind to the analyst. A minimum of 10 percent of all test samples must be QC specimens. Participation in internal and external proficiency testing surveys is required. Participation in an ADAMHA/NIDA-recognized accreditation and proficiency testing program for drugs of abuse is mandatory. The HHS Guidelines also contain mandated requirements for qualifications of laboratory directors, certifying scientists, and analysts. Among other requirements, a laboratory director must have documented scientific qualifications comparable to that of a person certified by the American Board of Forensic Toxicology or certified in Toxicological Chemistry by the American Board of Clinical Chemistry.

The NIDA Draft Standards for Accreditation of Laboratories Engaged in Drug Testing were under final review by NIDA in March 1987. These standards cover laboratory facilities, personnel, quality assurance and quality control, documentation of the testing process, and reports. Appendices pertain to a proposed laboratory inspection program and a proposed proficiency testing program for accredited laboratories. It was the NIDA plan to make accreditation under these standards a voluntary act at the discretion of drug-use testing laboratories rather than to accomplish it through licensure or registration by a federal regulatory agency. The purpose of the draft standards was described thus: "The National Institute on Drug Abuse proposes to establish minimum requirements to be met by any program designed to accredit laboratories engaged in urine drug testing for legal, regulatory, safety, employment or other non-medical purposes." Among key provisions of these draft standards were four relating to proficiency testing: 1) Positive results of initial (screening) tests must be confirmed by acceptable quantitative confirmatory procedures; 2) no false-positive drug identifications are acceptable; 3) at least 90% of all drugs for which service is claimed must be detected, i.e., a false-negative result rate of more than 10% is not acceptable in proficiency testing; 4) laboratories may be subject to proficiency testing. When these NIDA draft standards are promulgated in final form, they are likely to become the operational criteria or yardstick against which drug-use testing laboratories and drug-use testing activities will be measured, whether or not the laboratories seek accreditation under that program. All testing of federal employees will, of course, have to be performed in accredited laboratories, under the HHS Guidelines.

A potentially troublesome issue is whether, how, and what extent to accredit or regulate drug-use testing operations conducted "in-house" by industrial organizations and other private parties on persons within their respective organizations and enterprises. To the extent that drug-use testing is said by such parties to be at least partly justified by considerations of workplace safety and security and the public interest, one might analogize a requirement for conformity of such internal drug-use testing activities and programs to the existing statutory requirements for compliance with national fire protection codes, electrical codes, with regulations concerning procurement, storage, use and disposal of controlled dangerous substances or otherwise hazardous substances, and with the laws governing medical services provided within such private organizations. The choice of whether to engage in drug-use testing should be left to the private entities concerned, unless otherwise controlled by applicable law, regulations, contracts or other preemptive authority. However, if any such private party (actually many "private" parties are quasi-public in many respects by virtue of size, impact on the public interest, welfare, or safety, number of persons affected, or other attributes) elects to engage in drug-use testing it should be required by law to conform to statutory or other recognized and equivalent standards. That requirement for conformance should apply equally to testing conducted "in-house" and to testing externally conducted by others on behalf of the private party. This is one instance where exception from regulation and scrutiny because of small testing volume or other characteristics of the operation is no more justified than is exemption from child labor or minimum wage laws or from medical quarantine.
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208. This requirement of the HHS Guidelines coincides with recommendations made by the author at a December 13, 1983 White House/Department of Defense National Institute on Drug Abuse Conference on Military Drug Testing Programs that a ratio of at least 1 to 9 or 1 to 10 be maintained for quality control versus unknown specimens in urine drug-use testing. See Office of the Assistant Secretary of Defense, Minutes of the White House, DoD, NIDA Conference on Military Drug Use Testing Program 13 December 1983, Old Executive Office Building, at 12.

209. See Draft Standards, supra note 145.

210. Id.

211. See Scientific and Technical Guidelines, supra note 207.
B. Criteria for Laboratory Accreditation or Licensure

Regulation of drug-use testing can take various forms. Among these are accreditation and licensure, which would apply to laboratories and other establishments engaged in drug-use testing. Other mechanisms are available to evaluate personnel engaged or proposing to become engaged in drug-use testing, but it seems unlikely that in today's anti-regulatory climate schemes other than voluntary certification of personnel would succeed. For clarity, the three enumerated processes are defined as follows in the context of drug-use testing:

**Accreditation** — The process by which a governmental agency or nongovernmental organization evaluates a laboratory or other establishment engaged in drug-use testing, or a program of drug-use testing, and recognizes ("accredits") those entities which voluntarily seek accreditation and which meet predetermined qualifications, criteria or standards.

**Licensure** — The process by which a governmental agency grants permission to a laboratory or other establishment to engage in drug-use testing upon satisfaction of predetermined conditions and requirements. (A corollary to success of licensure programs is that the licensure legislation must prohibit conduct of the licensed activity by other than licensed establishments.)

**Certification** — The process by which an independent nongovernmental body grants recognition ("certification") to voluntary applicants who have met predetermined qualifications and requirements for such status in such respects as education, training, experience, and satisfactory performance on written and/or practical examinations.

It is obvious that interlocking relationships can develop between these different forms of recognition for all or some segments of a system meeting societal needs. Some current examples of entities seek what related to the field of drug-use testing which are encompassed by these three processes are: Qualifying educational programs for several scientific, biomedical (e.g., clinical chemistry) and medical professions are accredited by nongovernmental bodies; certain clinical laboratories are accredited by the College of American Pathologists under its Laboratory Accreditation Program, conferring "deemed status" equivalent to government licensure in some regards; and hospitals are accredited by the Joint Commission on Accreditation of Hospitals. Clinical laboratories, especially "independent" clinical laboratories (i.e., those not operating as an integral part of a hospital) are licensed by federal agencies and by certain states; licensure also exists in all states for physicians and certain other health-care practitioners and in some states for clinical laboratory personnel at various levels of responsibility. Certification is a widespread form of credentialing for health-care practitioners, including physician-specialists, medical technologists, bioanalysts, clinical chemists and other clinical laboratory scientists, as well as for such forensic scientists as forensic toxicologists, and for forensic pathologists.

Whatever form of professional or governmental regulation evolves for drug-use testing laboratories, it should have certain features if it is to be adequately effective in safeguarding the public interest and the interests of both the tested persons and those who utilize and rely upon drug-use testing in industry and elsewhere. First and foremost, the regulatory system should encompass essentially all drug-use testing, wherever performed. An exception could be made for drug-use testing performed exclusively for medical purposes, to the extent that it is equivalently regulated under other existing federal and state coverage of clinical laboratory activities. To regulate only some drug-use testing programs and laboratories and to exempt from regulation others on such grounds as location, test volume, testing technology, or initial use to be made of the results is to invite endless trouble, strife, inequalities, and litigation over such issues as denial of due process and denial of equal protection of the laws. Next, the universal regulatory system should be imposed and required by law, preferably by federal law analogous in applicable regards to the Clinical Laboratories Improvement Act of 1967 and its implementing regulations.212 Licensure or other authority to engage in drug-use testing should be conditioned upon documented initial compliance with a comprehensive and rigorous set of qualifications and requirements and upon documented continuing compliance with those criteria. Periodic inspections should be required at intervals of one year or less, carried out by qualified inspectors with appropriate professional qualifications, suitable training for those specialized inspections, and established absence of conflicts of interest. Deficiencies thus discovered must be promptly and fully remedied. Continued participation in one or more approved proficiency testing programs should be required for licensure, as should continued satisfactory PT performance accordance to preestablished and periodically reviewed or revised criteria. Blind proficiency testing, as previously discussed in this article, should also be a required part of the PT program.

212. See Clinical Laboratories Improvement Act, supra note 131.
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Licensure — The process by which a governmental agency grants permission to a laboratory or other establishment to engage in drug-use testing upon satisfaction of predetermined conditions and requirements. (A corollary to success of licensure programs is that the licensure regulation must prohibit conduct of the licensed activity by other than licensed establishments.)

Certification — The process by which an independent nongovernmental body grants recognition (“certification”) to voluntary applicants who have met predetermined qualifications and requirements for such status in such respects as education, training, experience, and satisfactory performance on written and/or practical examinations.

It is obvious that interlocking relationships can develop between these different forms of recognition for all or some segments of a system meeting societal needs. Some current examples of entities somewhat related to the field of drug-use testing which are encompassed by these three processes are: Qualifying educational programs for other scientific, biomedical (e.g., clinical chemistry) and medical professions are accredited by nongovernmental bodies; certain clinical laboratories are accredited by the College of American Pathologists under its Laboratory Accreditation Program, conferring “deemed status” equivalent to government licensure in some regards; and hospitals are accredited by the Joint Commission on Accreditation of Hospitals. Clinical laboratories, especially “independent” clinical laboratories (i.e., those not operating as an integral part of a hospital) are licensed by federal agencies and by certain states; licensure also exists in all states for physicians and certain other health-care practitioners and in some states for clinical laboratory personnel at various levels of responsibility. Certification is a widespread form of credentialing for health-care practitioners, including physician-specialists, medical technologists, bioanalysts, clinical chemists and other clinical laboratory scientists, as well as for such forensic scientists as forensic toxicologists, and for forensic pathologists.

Whatever form of professional or governmental regulation evolves for drug-use testing laboratories, it should have certain features if it is to be adequately effective in safeguarding the public interest and the interests of both the tested persons and those who utilize and rely upon drug-use testing in industry and elsewhere. First and foremost, the regulatory system should encompass essentially all drug-use testing, wherever performed. An exception could be made for drug-use testing performed exclusively for medical purposes, to the extent that it is equivalently regulated under other existing federal and state coverage of clinical laboratory activities. To regulate only some drug-use testing programs and laboratories and to exempt from regulation others on such grounds as location, test volume, testing technology, or initial use to be made of the results is to invite endless trouble, strife, inequalities, and litigation over such issues as denial of due process and denial of equal protection of the laws. Next, the universal regulatory system should be imposed and required by law, preferably by federal law analogous in applicable regards to the Clinical Laboratories Improvement Act of 1967[212] and its implementing regulations.[213] Licensure or other authority to engage in drug-use testing should be conditioned upon documented initial compliance with a comprehensive and rigorous set of qualifications and requirements and upon documented continuing compliance with those criteria. Periodic inspections should be required at intervals of one year or less, carried out by qualified inspectors with appropriate professional qualifications, suitable training for those specialized inspections, and established absence of conflicts of interest. Deficiencies thus discovered must be promptly and fully remedied. Continued participation in one or more approved proficiency testing programs should be required for licensure, as should continued satisfactory PT performance according to preestablished and periodically reviewed or revised criteria. Blind proficiency testing, as previously discussed in this article, should also be a required part of the PT program.

212. See Clinical Laboratories Improvement Act, supra note 131.
Essentials for drug-use testing laboratories were recently proposed by Dubowski and McBay.214 They comprise requirements, qualifications, guidelines, and safeguards in six categories: Facilities, personnel, methodology, quality assurance, documentation, and interpretation of analysis results. Together, these essentials constitute a set of acceptability criteria for drug-use testing laboratories and operations and for the results they produce. The specifics include laboratory director qualifications (DABFT or equivalent status) and mandatory confirmation of all presumptive tests and all immunoassay results by methods employing different chemical principles. The proposed essentials require the capability to perform both presumptive (screening) and confirmatory tests in the same laboratory facility, retention of all specimens yielding positive test results under proper storage conditions (e.g., in the frozen state at -20°C or lower temperature), access to all confidential information limited to a "need to know" basis, and result verification and interpretation carried out by persons with stipulated qualifications. These essentials could form the basis for qualifications, requirements, and procedures for governmental licensure or for nongovernmental accreditation of drug-use testing laboratories.

A national voluntary laboratory accreditation program (NVLAP) for private and public testing laboratories which serve regulatory and nonregulatory product evaluation needs was first established by the U.S. Department of Commerce effective February 25, 1976215 and is currently administered by the National Bureau of Standards. The organizational, administrative and technical details set forth in the current version of the Federal regulations pertaining to NVLAP216 affords a useful overview of many aspects of the organization and conduct of voluntary accreditation for drug-use testing laboratories. Other voluntary laboratory accreditation programs are currently operated by nongovernmental organizations in such fields as industrial hygiene, blood banking, and many others.

Another set of useful guidelines to serve as a starting point for regulation of drug-use testing is the pair of positions adopted by the


216. 15 C.F.R. Part 7.

American Chemical Society, the American Association for Clinical Chemistry, and the American Institute of Chemists on "Principles of Legislation and Rule Making for Regulation of the Practice of Clinical Chemistry"217 and "Principles of Proficiency Testing in Clinical Chemistry."218

The single most important aspect of any scheme for accrediting or regulating drug-use testing and drug-use testing laboratories is the element concerning laboratory staff and personnel. Because of their singular importance, standards for such personnel will be considered separately.

C. Standards for Personnel of Drug-Use Testing Laboratories

Drug-use testing, as currently performed, is a highly labor-intensive activity. The reliability of testing and the validity of the results are very dependent upon the qualifications, experience and judgment of the analyst and other professional and technical personnel concerned with a given biological specimen and with the testing system as a whole. To a far greater extent than in most commonly performed clinical chemistry laboratory tests, drug-use tests are non-automated and are performed individually or in sets or batches by partly or entirely manual methods which are often locally modified from their original published version, if any. The occasionally used term "urinalysis for drugs" is an unfortunate misappellation. Urinalysis is the performance of clinical laboratory tests of limited scope and complexity on urine specimens. A complete routine urinalysis typically consists of observation of the color, odor and appearance of the urine specimen, measurement of its specific gravity and reaction (pH), performance of simple tests for presence of excessive bile pigments, glucose, ketones or hemoglobin, and microscopic examination of the urinary sediment for evidence of blood cells, bacteria, crystals and other formed elements. Urinalysis is traditionally, if perhaps unfortunately, performed in clinical laboratories by the lowest ranking and least educated and trained laboratorians. More complex examinations on urine are never designated as "urinalysis," but are simply given the applicable microbiological, clinical, chemical or other designation pertinent to the analyze or procedure of interest, e.g.


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bacterial culture, hormone analysis, etc.; and they are performed in the corresponding clinical laboratory unit. The fact that the specimen most commonly used in drug-use testing at present is urine does not alter the status of these examinations as toxicological analyses and does not make such urine drug-use testing "urinalysis" in the traditional sense of that term. Hence, the applicable personnel standards are those for the former, not the latter kind of laboratory service.

Fortunately, the consensus of informed opinion on the necessary qualifications of laboratory personnel engaged in drug-use testing is coalescing. Several of the recently promulgated federal guidelines, i.e., the HHS Guidelines\(^{219}\) and the NIDA Draft Standards,\(^{220}\) incorporate substantially the same requirements for laboratory directors and real interpreters/certifying officials as have been proposed by the profession.\(^{221}\) To obviate the need to delineate or reconcile minor differences in the necessary qualifications of laboratory personnel set forth in the several foregoing sources, the following material is represented only as this author’s views of the necessary personnel standards for drug-use testing.

**Director.** The laboratory (scientific) director should (1) have an earned doctoral degree (Ph.D., or Sc.D.) in chemical science, pharmacology, or toxicology, from an accredited institution; and (2) hold current certification in Forensic Toxicology by the American Board of Forensic Toxicology or certification in Toxicological Chemistry by the American Board of Clinical Chemistry.

In lieu of the above certification,\(^{222}\) the laboratory or scientific director must possess documented qualifications equivalent to those required for such certification by ABFT or ABCC, except that only two years of acceptable full-time professional experience in forensic toxicology, toxicological chemistry, or analytical toxicology (or the part-time equivalent thereof) should be required. The director must also possess adequate and appropriate training and/or experience in the forensic aspects of analytical toxicology. The professional experience should include at least two years of experience in the analysis of biological specimens for drugs of abuse.

**Supervisor.** Supervisors of analysts should have (1) an earned baccalaureate or higher degree in chemical science from an accredited institution; and (2) at least two years of acceptable full-time training and experience in analytical toxicology, toxicological chemistry or forensic toxicology (or the part-time equivalent thereof); and (3) theoretical and practical training in the technology of drug-use testing in use in the employing laboratory, together with an adequate understanding of quality assurance and quality control concepts and procedures.

In lieu of qualifications (1) and (2), supervisors must (1) possess an earned baccalaureate or higher degree in medical technology or in physical or biological sciences, and hold current registration as a Registered Medical Technologist, MT(ASCP), by the American Society of Clinical Pathologists, or hold current certification as a Clinical Laboratory Scientist, CLS, by the National Certification Agency for Medical Laboratory Personnel, or hold formal recognition which is the documented equivalent of the foregoing; and (2) have had at least two years of acceptable full-time experience in clinical chemistry or other relevant clinical laboratory discipline (or the part-time equivalent thereof).

**Analyst:** Other laboratory analysts or technicians should possess education, training and skills commensurate with the tasks performed. Those tasks and functions should involve only limited exercise of independent judgment, and should not be performed by analysts or technicians in the absence of a qualified supervisor.

The personnel of drug-use testing laboratories should also possess documented good moral character and appropriate professional or technical competence, and be eligible for the appropriate level of access to controlled dangerous substances under applicable federal and state laws and regulations. To maintain and enhance the skills of persons engaged in drug-use testing activities, adequate in-service training and continuing education programs of appropriate levels and frequency should be conducted within the laboratory for all such personnel or be otherwise available to them. Personnel files containing complete records should be maintained within the laboratory and should include at least the following information: Full identification (name, social security number, photograph), record of education (with degrees) and prior experience, certifications or licenses, references, detailed job description, pertinent health and job safety records, chronological record of performance evaluations, advancements and promotions, results of tests for color

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220. See Draft Standards, supra note 145.
221. See Dubowski & McBey, supra note 214.
222. The two leading certifying bodies in relation to drug-use testing, which are enumerated in the Federal HHS Guidelines and NIDA Guidelines, are The American Board of Forensic Toxicology (225 South Academy Blvd., Colorado Springs, CO 80910) and The American Board of Clinical Chemistry (c/o Dr. William H. Fenn, Department of Pathology, University of Kentucky Medical Center, Lexington, KY 40536) with respect to its certification program in Toxicological Chemistry.
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blindness,\textsuperscript{223} and pertinent incident reports.

Whether to perform or require drug-use testing of laboratory personnel engaged in drug-use testing is a delicate problem on which there is no unanimity at present. It has unfortunately been documented that drug abuse, drug misuse, and diversion or drugs and controlled substances from the workplace occur occasionally among the staff of clinical and forensic laboratories, as they do among health care practitioners. If these laboratory personnel are subjected to drug-use testing, it should be carried out with all necessary safeguards and the testing itself should be performed in a laboratory other than that in which the person is employed. Interpreters of the results of such tests should be aware that legitimate occupational exposure to drugs of abuse under some circumstances can lead to inadvertent intake, for example by inhalation of cocaine dust and other atmospheric contaminants during the handling of large quantities of controlled substances by inadequately-protected analysts.

VIII. Conclusions and Recommendations

The foregoing information and considerations and this author’s experience in drug-use testing give rise to the following conclusions and recommendations. For ready future reference, they have been numbered, but the numerical order does not necessarily indicate relative importance or priority.

A. Conclusions

1. Drug-use testing for non-medical purposes is a large and growing field which is presently largely unregulated, but which should be subject to universal and uniform national regulation.

2. Non-medical drug use testing is, in essence, a forensic science activity.

3. The existing technology for analysis of drugs and drug metabolites of interest in drug-use testing is, under proper conditions, capable of yielding correct analysis results validly reflecting the absence or presence, identity and concentration, of target analytes. However, all procedures and methods for drug analysis are subject to error, especially occurrence of random errors.

4. The interpretation of the results of drug-use testing is less advanced than the best analytical procedures, especially with respect to establishing the relevance and significance of those results.

5. In general, drug-use testing as currently practiced, when urine is the tested biological specimen, does not provide information about past or present patterns of drug use, abuse, or drug-dependence, or about drug-related mental or physical impairment, or other effects, at any given time.

6. There is urgent need for up-to-date, authoritative and comprehensive information about non-medical drug-use testing and the analytical methodology available for that purpose. Periodic updating of such information will also be needed.

B. Recommendations

1. A comprehensive and universal nationwide system of regulation of non-medical drug-use testing should be established forthwith, preferably in the form of federal licensure with provisions for alternative accreditation under standards identical to those for federal licensure. All non-medical testing in the public and private sectors should be subject to this regulatory system. Regulation of drug-use testing should specifically apply to both laboratories and other establishments engaged in drug-use testing and to personnel; and it should include adequate and appropriate proficiency testing, inclusive of blind proficiency testing.

2. Non-medical drug-use testing activities should be conducted under established standards applicable to other forensic science activities.

3. Both medical and non-medical testing should include appropriate testing for ethyl alcohol.

4. All persons subjected to non-medical drug-use testing should be informed of the fact beforehand, and be provided with full information concerning the nature and extent of the tests to be conducted (including enumeration of all target analytes) and the testing technology employed. A copy of all results obtained and provided to any other party should also be promptly provided to the tested person. Surrogate testing and clandestine testing of biological specimens for drugs and/or metabolites should be prohibited statutorily; and all drug-use testing should
blindness, and pertinent incident reports.

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223. Color blindness tests are indicated for personnel involved in analysis and verification aspects of drug-use testing because recognition and differentiation of the color of specimens, spots on thin-layer chromatograms, etc. play an important role in the testing process and affect the analysis outcome. Abnormal color vision occurs in about 8% of males and about 0.4% of females. Analysts and their supervisors need to be aware of instances of color blindness.
be conditioned upon properly obtained informed consent, except as otherwise provided by law or ordered by a court of competent jurisdiction.

5. Records and reports of the results of non-medical drug-use testing should exclude incidental information arising in the course of the testing, whether sought or fortuitously discovered, on such matters as the state of health of the tested person, apparent existence of disease or infirmity, presence and identity of drugs and medicaments other than those on the official preestablished list of analytes, oral contraception, or any other personal information.

6. All biological specimens which have yielded positive results for drugs and/or drug metabolites should be retained in the laboratory, under conditions adequate to prevent substantial change or deterioration, for at least one year or until final disposition of any proceeding arising from such results, whichever is later.

7. At the time of initial collection of urine or other biological specimens for drug-use testing, a separate specimen aliquot should be obtained, properly identified, and suitably and securely stored. It should be of adequate quantity and quality for any subsequent independent analysis in the event of challenge to the results of drug-use testing to be performed upon the main specimen. This reserve aliquot is in addition to and is not a substitute for retention of the remaining portion, if any, of any specimen which yields positive results in drug-use testing. It provides a separate safeguard against intentional or unintentional use, or loss of, the entire main specimen during the course of drug-use testing. Its collection and retention is probably the single most important safeguard in the entire drug-use testing process.

8. Laboratory reports of the results of drug-use testing should state, at a minimum: (a) Information uniquely identifying the specimen to which the report pertains, whether by number, name or otherwise; (b) the drugs, drug metabolites or other target analytes encompassed in the testing process; (c) the type, analytical principle, and methods of all tests conducted for initial (screening) and for confirmatory testing; (d) the cutoff concentration or other quantitative test criterion employed for each substance encompassed among the target analytes, used to establish whether the result is positive or negative; (e) the results or findings for each test conducted; (f) the respective dates of receipt and analysis of the specimen; (g) the name(s), title(s) and other necessary identifying details for the persons who conducted the testing of the specimen and the review and validation of the reported findings; (h) the date of issue of the laboratory report. In the event of actual or suspected inadequacy of the specimen noted (e.g., evidence of decompo-

9. All non-medical drug-use testing should mandatorily require adequate and appropriate independent confirmation of all positive findings of initial, preliminary, presumptive, or screening tests, by confirmatory tests which employ different chemical principle(s) from that used for initial testing and which possess specificity and sensitivity (as defined herein, supra) at least equal to those of the initial testing methods. In selected situations, negative initial test results should also be comparably confirmed.

10. Cutoff concentrations or other qualitative and quantitative analysis criteria used to establish whether results of testing or analysis for a given drug and/or drug metabolite are to be considered positive or negative, should be nationally standardized. This will promote uniformity of reporting of the results of drug-use testing and foster the use of identical or at least comparable means of assessing the significance of the analysis findings. Ideally, the same reported result should mean the same thing, wherever the testing took place or the results were interpreted.

11. The pertinent characteristics of analysis methods set forth in Table 3, especially those affecting the reliability of the method, should be experimentally determined in each laboratory for every analysis or test method in use in that laboratory, prior to initial use of any method and at appropriate intervals thereafter not exceeding one year. Complete records of the method evaluations should be maintained in the laboratory and be readily available upon request by any appropriately interested party.

12. The inherent limitations of urine as a specimen for drug-use testing should be widely publicized and be fully recognized and appreciated by persons responsible for establishing and conducting drug-use testing programs, for conducting tests and analyses upon urine specimens, and for interpreting the results of such tests. In particular, steps should be taken to achieve universal recognition of the fact that drug-related impairment or drug influence can neither be established nor presumed from the results of urine testing for drugs and/or drug metabolites, nor the dose and time of drug intake or exposure be established or presumed from such results with adequate validity and certainty for legal and quasi-legal purposes.

13. Whenever urine is used as a biological specimen for drug-use testing, all quantitative analysis results and all cutoff concentrations
be conditioned upon properly obtained informed consent, except as otherwise provided by law or ordered by a court of competent jurisdiction.

5. Records and reports of the results of non-medical drug-use testing should include incidental information arising in the course of the testing, whether sought or fortuitously discovered, on such matters as the state of health of the tested person, apparent existence of disease or infirmity, presence and identity of drugs and medicaments other than those on the officially preestablished list of analytes, oral contraceptives, or any other personal information.

6. All biological specimens which have yielded positive results for drugs and/or drug metabolites should be retained in the laboratory, under conditions adequate to prevent substantial change or deterioration, for at least one year or until final disposition of any proceedings arising from such results, whichever is later.

7. At the time of initial collection of urine or other biological specimens for drug-use testing, a separate specimen aliquot should be obtained, properly identified, and suitably and securely stored. It should be of adequate quantity and quality for any subsequent independent analysis in the event of challenge to the results of drug-use testing to be performed upon the main specimen. This reserve aliquot is in addition to and is not a substitute for retention of the remaining portion, if any, of any specimen which yields positive results in drug-use testing. It provides a separate safeguard against intentional or unintentional use, or loss of, the entire main specimen during the course of drug-use testing. Its collection and retention is probably the single most important safeguard in the entire drug-use testing process.

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9. All non-medical drug-use testing should mandatorily require adequate and appropriate independent confirmation of all positive findings of initial, preliminary, presumptive, or screening tests, by confirmatory tests which employ different chemical principle(s) from that used for initial testing and which possess specificity and sensitivity (as defined herein, supra) at least equal to those of the initial testing methods. In selected situations, negative initial test results should also be comparably confirmed.

10. Cutoff concentrations or other qualitative and quantitative analysis criteria used to establish whether results of testing or analysis for a given drug and/or drug metabolite are to be considered positive or negative, should be nationally standardized. This will promote uniformity of reporting of the results of drug-use testing and foster the use of identical or at least comparable means of assessing the significance of the analysis findings. Ideally, the same reported result should mean the same thing, wherever the testing took place or the results were interpreted.

11. The pertinent characteristics of analysis methods set forth in Table 3, especially those affecting the reliability of the method, should be experimentally determined in each laboratory for every analysis or test method in use in that laboratory, prior to initial use of any method and at appropriate intervals thereafter not exceeding one year. Complete records of the method evaluations should be maintained in the laboratory and be readily available upon request by any appropriately interested party.

12. The inherent limitations of urine as a specimen for drug-use testing should be widely publicized and be fully recognized and appreciated by persons responsible for establishing and conducting drug-use testing programs, for conducting tests and analyses upon urine specimens, and for interpreting the results of such tests. In particular, steps should be taken to achieve universal recognition of the fact that drug-related impairment or drug influence can neither be established nor presumed from the results of urine testing for drugs and/or drug metabolites, nor the dose and time of drug intake or exposure be established or presumed from such results with adequate validity and certainty for legal and quasi-legal purposes.

13. Whenever urine is used as a biological specimen for drug-use testing, all quantitative analysis results and all cutoff concentrations

https://nsuworks.nova.edu/nlr/vol11/iss2/22
should be “normalized” for the creatinine concentration of the urine specimen under examination. In practice, this means that all drug or drug metabolite concentrations, or other numerical data for cutoff values or other purposes, should be determined and expressed in terms of the analyte concentration or other value per milligram of creatinine present in the urine specimen under examination (established by chemical measurement) instead of being expressed per milliliter of urine or other volume indicator.

14. Systematic and organized research efforts should be mounted to select, evaluate, and validate other biological materials to replace urine as a specimen for drug-use testing. In particular, both pooled and mixed saliva should be investigated as potentially preferable alternative specimens.

Urine Testing for Drugs

Karen Hudner*

Introduction

Early in May 1985, the ACLU made headlines in the sports pages of The Boston Globe. “ACLU Calls Drug Plan ‘Invasion of Privacy’” shouted the caption from the top of the page.1 Such noteworthy attention in the sports pages is probably a first for the organization. Ira Glasser, baseball fan extraordinary, Brooklyn Dodgers fanatic and ACLU national director, had assailed Peter Ueberroth’s new drug testing plan as an invasion of privacy. Glasser noted that, “The question it raises is whether or not it is permissible to invade the privacy of thousands who are innocent of drug use in order to find a handful of drug users . . . . There’s an old Southern song — if you hang ‘em all, you get the guilty.”2

Ueberroth’s edict was a well-publicized part of a growing trend among employers to require urine tests for drugs as a condition of employment. In Massachusetts, the first most widely reported effort to institute a drug testing program occurred in the Spring of 1984. Boston Edison, an employer of approximately 4100 people, began requiring the tests as part of a pre-employment physical as well as a pre-condition to transfers or promotions.3 Likewise, in New Jersey4 and Arkansas,5 school boards had voted to require blood and urine tests for students and on Long Island6 teachers seeking tenure were required to submit urine samples. Furthermore, about 30 percent of all Fortune 500 com-

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1. The Boston Globe, May 9, 1985, at 56.
2. Id.
5. Judge Franklin Waters entered a judgment on July 12, 1985, and modified it on September 5, 1985, against the Arkadelphia School District’s use of urine tests to determine whether to expel students suspected of using drugs. Judge Waters found the urine tests excessively intrusive. See Arkadelphia School Dist.’s Use of Urine Test Ruled Unconstitutional, 17 ACLU Newsletter of Arkansas 1 (1985).