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Urine Testing for Drugs of Abuse

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Urine Testing for Drugs of Abuse

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Urine Testing for Drugs of Abuse

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Foreword

In the past 5 years, a growing concern over the use of illicit drugs in the workplace has led to an interest in urinalysis as a way to detect and deter drug use. Drug testing by urinalysis has been suggested and in many cases implemented for prospective and current employees in industry; for personnel of the armed forces; for parolees and bail seekers in civilian court systems; for workers in the transportation industry; and for individuals who serve as role models, such as nationally known athletes. Two factors have led to the widespread use of urinalysis for drugs: technical developments in testing methods and the growing demand for drug testing. Society is becoming increasingly aware of the impact of drug use on public safety and of the financial impact on industry of lost time and productivity. The annual loss of productivity of employees has been estimated at $100 billion for alcohol and drug abuse, a third of which is due to drug abuse alone.

Drug and alcohol abuse in the workplace is amenable to carefully planned prevention programs, however, and urine drug detection provides a powerful tool for use in such programs. Preemployment urine screening is now common among "Fortune 500" companies and in several Federal agencies.

As a consequence of drug screening programs, laboratories that were established to perform urinalysis associated with methadone treatment have had to greatly expand their capacities; many new laboratories have sprung up to meet the demands for drug assays; and clinical laboratories associated with medical centers, under economic pressures in recent years, have begun to venture into drug testing. However, results from laboratories that are not subject to any established guidelines for drug testing are sometimes unreliable. At present few guidelines exist for private laboratories; the Department of Defense has strict certification requirements for laboratories testing military personnel, and the Federal Railroad Administration as well as State agencies in California and New York have quality control standards in place, the latter for laboratories associated with methadone treatment programs. Until quality control programs are mandated on a broad scale, however, employers wishing to establish a drug screening program must rely on their own initiative to evaluate the reliability of a testing laboratory, so that no individual will be falsely accused of drug use and at the same time regular use on the part of any tested employee will not escape detection.

The National Institute on Drug Abuse (NIDA) is in a unique position to provide advice on many of the technical issues associated with the role of urinalysis in the prevention of drug abuse. Since 1972, this Institute has supported major research efforts to develop analytical methods for detecting and measuring drugs in biological fluids, with special emphasis on methods for cannabinoids and other abused drugs. NIDA has supported the development of many of the major technologies beiug used today for urine drug screening and confirmation. NIDA has also played a leading rote in the study of behavioral and pharmacokinetic effects of drugs of abuse--two areas of knowledge that are critical to the assessment and implementation of effective drug screening programs.

The purpose of this monograph is to provide informatiou that will assist those involved in the planning or implementation of drug testing programs in making informed choices: information such as what urine screening can and cannot do, how it fits into an overall drug program, and how it can be used most reliably. It is our hope that this powerful technology which has grown out of basic research programs will be used to advantage to assist in the prevention of drug abuse in the United States.

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Establishing a Urinalysis Program-Prior Considerations

Richard L. Hawks, Ph.D.

Urinalysis for detection of drug use should be considered in the context of an overall plan to reduce or prevent the negative impact of drug abuse on an industry or organization. It would be inadvisable, however, to proceed without a careful assessment of the group to be affected by testing. Certain questions should be addressed: What personnel, if any, are affected by drug use? Which drugs are the major problem? Can the organization shoulder the economic burden for an appropriate urinalysis and counseling program, and perhaps the legal and emotional costs associated with such a program?

In some cases, a problem is obviously present, based on incidents of known drug use or drug dealing within the organization. Or it may be only suspected, due to higher than normal rates of absenteeism, decreases in productivity, or an increase accidents or thefts in the workplace. In the absence of any indicators of drug use, managers might justifiably decide to pursue the issue no further.

Assessment starts with education. Managers of a potential drug program should first become knowledgeable themselves about drug abuse and its indicators. Information is available from State drug and alcohol abuse organizations, through the National Clearinghouse for Drug Abuse Information at NIDA (P.O. Box 416, Kensington, MD 20795), through forensic science centers associated with universities or medical examiners' offices, and from a number of expert private consultants.

The presence and extent of a drug problem could be assessed by means of a survey based on anonymous questionnaires. This approach could include an anonymous urine drug screen that would not only let the management know what to expect if a regular urinalysis program were initiated; it would also constitute a "dry run" with few legal ramifications, and would send a clear message to employees about the seriousness of concern about the drug problem and the resolve to remedy it. This could in itself have a deterrent effect.

Any assessment should include clarification of which substances are involved. Alcohol is the largest problem in most industries; an estimated 100 million Americans are current users of alcohol. The most recent NIDA survey estimates that 18 million Americans are current users of marijuana and 5.8 million are current users of cocaine (based on admitted use at least once during the month prior to the survey). In some locations, including Washington, DC, and Los Angeles, use of phencyclidine (PCP) is particularly acute. These illicit drugs, along with opiates, amphetamines, or hallucinogenic compounds, have a significant presence in our society.

A more complicated group of drugs to assess are the prescription drugs; tranquilizers, barbiturates (sleeping pills), and antidepressants are probably even more prevalent in the workplace than illicit drugs and potentially are just as likely to impair job performance or to create health problems if used in excess of prescribed amounts or without adequate medical supervision.

When the "assessment" questions are resolved, establishing an appropriate drug abuse deterrent program, including urinalysis, becomes feasible. The care with which the plan is developed and implemented will determine the success of the program and associated benefits. The plan should include careful consideration of the organization's policies, goals, and philosophy. Finally, the plan should be well documented and available to all employees of the organization.

The plan should be tailored to the extent of the problem. If no clear indication of significant drug use at a worksite or in an organization is apparent, a program beyond a preventive educational effort may not be warranted. Evidence of heavy psychoactive drug use in job situations where safety is a sensitive issue should be dealt with more aggressively. The plan should give paramount consideration to the purpose of the program, the essence of which is usually the health and safety of employees as well as economic concerns. Another important element of the plan should be to guarantee the personal privacy and dignity of the employees as much as possible.

Various alternatives to a urine testing program may be considered. If the problem does not warrant a major program, it may be sufficient to maintain an awareness of drug abuse indicators, to define isolated problems regularly through the use of personnel data and supervisor reports, and to deal with them through an employee assistance program.

Most of the urine samples being analyzed in industry today are associated with preemployment applications. While many of the rights usually accorded an applicant are not necessarily the same as those of an employee, the same rights of privacy and accuracy of analysis should be accorded these individuals. A preemployment screening plan may preclude employment of individuals with positive urines, but such a plan should include some kind of counseling, not only to make the individual aware of why the job was denied but also to offer advice and direction in dealing with the drug problem. A more enlightened probationary policy might allow the hiring of such an individual based on other merits, on the condition that counseling be obtained and drug use cease.

Therapeutic drugs, while potentially problematic when abused, may in fact improve or enhance workplace safety by decreasing health-related impairment. Chronic disease states that would be problematic in the workplace or on the highway without the benefit of drugs include epilepsy and mental illness. Other conditions such as stress may also contribute to impaired performance and therefore benefit by drug therapy. Urine drug programs may identify those individuals who are legitimately taking drugs to alleviate these disease states. Consideration should be given to the fact that sufficient discrimination still exists in our society that individuals with these particular conditions may have been actively hiding them from their coworkers or employers. This further argues for confidentiality in the handling of urinalysis results, in the same way an organization would handle an individual's medical records.

Other drug-specific characteristics that might influence policy development are whether some drugs, because of their particular pharmacological effects, may be of more concern than others. Alcohol, marijuana, phencyclidine, heroin, and LSD, for instance, are all drugs of abuse, but they differ in the time course of their effects on performance and on the time their previous use can be detected. Marijuana components in some circumstances can be detected for weeks after the last use; indication of cocaine use can rarely be detected in urine for more than a day or two. A cocaine-positive urine therefore indicates recent and possibly dependent use, particularly if the individual was aware of the pending analysis and still could not abstain. A similar conclusion cannot be drawn from a single marijuana-positive urine.

Effective programs for the detection of drugs of abuse in human urine specimens are best accomplished with sensitive testing procedures. Because of the numerous legally sensitive features of drug detection programs, the analytical results must be unquestionably reliable and able to withstand considerable scrutiny. Therefore, the testing laboratory must be experienced and capable in a number of important functions, including quality control, documentation, chain of custody, technical expertise, and demonstrated proficiency over time in urinalysis testing. Most important, the laboratory must produce data that are secure from false positives and defensible in a forum where the data may be challenged (e.g., a legal hearing or subsequent arbitration). A qualified expert should be available to testify as to the laboratory procedures that were employed, as well as to the accuracy and reliability of the testing result.

At the moment, private laboratories providing urine drug detection services for private industry in the United States are generally not compelled to comply with specific guidelines associated with accuracy of analysis. Oversight of urinalysis laboratories is essential to ensure quality analyses and to provide public credibility, either mandated by government regulation or established within the private sector and coupled with motivation of the laboratories to participate in such oversight programs. Government and private industry appear to be interested in moving in this direction. Some State legislatures are considering bills to regulate drug urinalysis laboratories, and some organizations and companies are preparing to provide certification and proficiency-testing services to urinalysis laboratories. Performance and qualification standards that will be the basis of a NIDA registry of laboratories engaged in urine drug detection services are currently under development. The success of such programs will depend on the motivation of laboratories to participate in them--motivation that will ultimately come from the laboratories' clients who will demand the quality and performance for which they are paying. NIDA officials hope this monograph will provide sufficient background to enable both clients and employees to ask the right questions and demand the appropriate quality of analysis.

Many considerations are important to developing a sound drug program in an organization. It cannot be overemphasized that the documentation of well-thought-out policies, developed with input from all organizational elements, is at the top of the list. An effective program to discourage drug abuse in an organization must have clearly defined rationales, goals, and rules. The consequences of a positive urinalysis result must be clearly stated and not open to arbitrary responses by management. The rights and sensitivities of the individual should be protected as much as possible. Results of urine drug assays should be kept confidential. The individual

should be accorded the benefit of trained counseling, with referral to treatment programs if necessary. The program should be designed, in other words, for prevention and rehabilitation rather than for law enforcement.

A technically effective program that generates negative results can be considered successful, one that is serving to prevent drug use by employees and simultaneously to provide management (or the public) with assurance that alcohol and illicit drugs at least are not a factor in performance problems in the organization. Positive results represent failures of the prevention system. Nevertheless, as long as they are looked at as diagnostic of a medical condition or an attitude that is susceptible to appropriate intervention or treatment strategies, the system will foster hope for improvement of the drug abuse situation in this country.

The chapters that follow will present examples of methods and procedures to ensure the quality and accuracy of test results. They will concentrate on the technical aspects of the methodology used for urinalysis, the means to ensure accuracy in such analyses, and the background to help in the interpretation of assay results. Descriptions of appropriately controlled and monitored urinalysis programs currently in use will be presented, as well as specific descriptions of what to look for in choosing a laboratory.

Although some parts of the text appear to be geared toward the scientist, the monograph has been written to inform readers in all phases of program operation. Some chapters discuss the practical aspects of conducting drug testing programs, including laboratory selection and quality assurance and examples of programs already underway. Others relate the technical concepts and methods involved in employing urinalysis to detect drug use. The final chapter summarizes testing approaches to selected drugs of abuse and offers reference lists for further study.

Drug Testing Programs

Robert E. Willette, Ph.D.

Many government agencies and private employers have initiated drug testing programs. Although the vast majority have started since 1981, many have been in place for several years. In order to appreciate the variety and scope of different approaches to drug testing, several programs will be described.

It can be noted that most of the programs include drug testing under one or more of the following conditions: (1) before employment or during a probationary period; (2) under reasonable suspicion of alcohol or drug use, such as following an accident or bizarre behavior; (3) as part of routine physicals, often required by Federal regulations; (4) during random testing; and (5) when monitoring employees during rehabilitation or counseling for drug use.

MILITARY SERVICES

By far the most extensive programs of drug testing are conducted by the U.S. military services. Of these, the U.S. Navy program is the most intensive and successful. The Navy operates five drug testing laboratories, through which approximately 1.8 million urine specimens are processed each year, each being tested at the present time for cannabinoids, cocaine metabolite, phencyclidine, amphetamines, opiates, and barbiturates. The Navy is currently planning to include testing for LSD. The 1.8 million specimens are collected, primarily on a truly random basis, at a rate of almost three times per year per member of the Navy and Marine Corps. Since it is random, some members may be required to provide specimens several times a year and some none. This serves as a constant deterrent to drug use.

The Army and Air Force also collect random specimens but at a lower frequency. AU of the services can collect specimens in the event of a probable cause situation, usually Limited to cases of suspected drug use. It is important to note that military standards for probable cause follow the strictest legal standards and cannot be easily abused. Some amount of testing is used for service members who have tested positive once and have completed counseling and/or rehabilitation programs. They are usually placed on a surveillance program for several weeks following their return to active service.

All of the military services collect specimens under direct observation. This is done to eliminate the possibility of specimen substitution or adulteration. Nevertheless, laboratories occasionally receive specimens that have been

adulterated. If it can be proven that the provider of the specimen did adulterate the specimen, the individual will be subject to other charges. Also, the observer can be disciplined for failing to perform his or her duty. In this manner, it has been possible for the military to maintain a tight and effective program.

The individual commanding officer can determine if the initial positive result is sufficient grounds to discipline or discharge a service member for wrongful ingestion of an illegal substance. In cases of a good service record and significant promise for continued useful service, the member can he retained. This is not very common following a second positive result.

The success of these programs can best be illustrated with the record of the Navy. Starting in 1981 with an indicated use level of 48 percent for enlisted personnel under the age of 25, the overall test results for any period after 1984 have been below the 5 percent screened-positive rate. Screening figures are used, since not all specimens are confirmed due to the presence of drug concentrations below the confirmation cutoffs. Admitted drug use in this same population was about 10 percent, as indicated in a questionnaire survey taken by the Department of Defense in 1985.

Standards for performance are monitored in the military program in several ways. Testing methods and cutoff levels are established by the Office of the Assistant Secretary of Defense for Health Affairs. That Office conducts an annual inspection of each military and contract laboratory. In addition, each service conducts regular inspections of its own laboratories. The Navy laboratories are inspected quarterly by the Naval Medical Command, which is responsible for the operation of the laboratories, and annually by a team of operations and legal personnel and outside civilian experts. All of the laboratories are also monitored by a proficiency testing and blind quality control program, as described in the next chapter.

ADMINISTRATIVE OFFICE OF THE U.S. COURTS

The Administrative Office of the U.S. Courts is responsible for people who are on probation or parole for Federal crimes. If this involved the use or possession of drugs, they are provided counseling, if necessary, and are monitored on a regular basis by means of urine testing. The Office contracts with commercial laboratories for testing and monitors their performance by submitting periodic blind quality control samples. Persons found positive for drug use (usually more than once) have to appear before a Federal judge to determine if probation or parole has been violated.

FEDERAL BUREAU OF PRISONS

In a manner similar to that of the U.S. Courts, the Federal Bureau of Prisons maintains a contract laboratory to test specimens collected from Federal prisoners. These are comprehensive drug screens that include a number of prescription drugs as well as the more commonly used drugs of abuse. Since drug administration is closely controlled, it is necessary to monitor for the use of prescription drugs that are obtained illicitly. It usually requires repeated offenses before prisoners have to appear before a judge to alter their sentences or to have certain privileges removed.

DEPARTMENT OF TRANSPORTATION

The U.S. Department of Transportation has regulatory authority over several transportation industries, for which it has established certain drug-related rules. In addition, the following DOT agencies have established drug testing requirements for their own employees.

Coast Guard

The U.S. Coast Guard is a uniformed service and maintains a drug testing program similar to that of the military services. Personnel are tested on a random basis and following probable cause incidents. The Coast Guard uses a contract laboratory to which specimens are sent under strict chain of custody. The frequency of testing varies from region to region but is at the rate of approximately once a year.

The Coast Guard has also issued proposed regulations for shipping fleets covered under merchant marine laws.

Federal Aviation Administration

It has been announced that, during 1986, the Federal Aviation Administration will initiate testing of all of its employees at the time of their required periodic physical examinations. The program will be managed under a contract that will conduct the testing as well as monitor collection of specimens by approved air flight surgeons. Appropriate counseling and/or treatment will be provided for any employee found to be using illegal drugs.

The FAA does not have any specific drug testing requirement for its regulated industry, commercial aviation. Regulations do lay out requirements for action following drug-related incidents, although the agency has announced that no drug-related commercial airplane crash has ever occurred. The physical examination requirements also preclude pilots who are diagnosed as being addicted to alcohol or drugs. It is not known if any airline tests its pilots for drugs, although many have announced that they screen job applicants for drugs.

Federal Railroad Administration

In 1985, the Federal Railroad Administration (FRA) reissued regulations governing railroad operations that included specific rules for drug testing. This "Control of Alcohol and Drug Use in Railroad Operations" rule follows a 10-year history of 48 alcohol- and drug-related train accidents and incidents that resulted in 37 fatalities, 80 injuries, and $34 million in damages. The new regulations, which did not take effect until February 1986 following several court actions, require preemployment drug testing and testing for alcohol and drugs following certain accidents and in reasonable cause situations. The regulations also spell out rather specific standards for the testing, including publication of a field manual to assist the railroads in developing their own programs.

National Highway Traffic Safety Administration

Under the Motor Carrier Safety Regulations, the National Highway Traffic Safety Administration has established physical qualification standards for drivers engaged in interstate highway activities, i.e., trucks and buses. One requirement has vacillated from "does not use amphetamine, narcotic, or any habit forming drug" to "has no current diagnosis of drug addiction." Although the regulations do not specifically require drug testing, many regulated companies have included drug testing as part of the DOT physicals, since this is the only reliable means of detecting drug use. The regulations also permit the discharge of a driver who has been found in possession of or "under the influence" of a drug while on company property or on duty. No standards have been established, although the regulations include all substances covered under Schedule I of the Controlled Substances Act.

REGULATED TRANSPORTATION INDUSTRIES

Many companies in the air, rail, bus, and trucking industries have implemented various drug testing programs, often to comply with the mentioned regulations or on a voluntary basis. Most common are preemployment tests, which are the easiest to conduct from all legal aspects. Only the Federal Railroad Administration has published standards for testing.

In a rather provocative and forward move, the International Brotherhood of Teamsters signed an agreement with the major trucking fiis that spells out specific standards and conditions for drug testing. These include collection procedures, test criteria, cutoffs for determining violations, and penalties. The agreement requires that employees receive 30 days' notice before they have to provide a urine specimen as part of their physical examination. This provides an opportunity for the employee to stop using drugs before being tested. There are also provisions for testing employees under "probable suspicion" situations, which would include accidents.

Several railroads implemented strict drug testing programs prior to the effective date of the FRA regulations. One company, Southern Pacific, has announced that human-factor accidents have gone down each year from 37 percent to 69 percent over the period of 1983 to the first 6 months of 1986 since implementing the testing program.

DEPARTMENT OF JUSTICE

During 1986, the Department of Justice authorized the implementation of broad drug testing programs for its agencies. Although no indication of drug use by its employees was seen, it was felt that the Department should set an example for other government agencies and private employers. Several of its agencies have tested employees in the past when there has been reasonable suspicion that the employees were using illegal drugs.

Drug Enforcement Administration

The Drug Enforcement Administration (DEA) is entrusted with enforcing the Nation's drug laws and, as such, felt it was important to demonstrate that

the agency was free of drug use. The program was initiated in 1986 in a progressive manner. Testing began with trainees and will gradually include all personnel, adding classes of employees in the more critical positions. Testing will be done on a random basis, with collection and testing conducted through a contract. The agency has an employee assistance program that is available on a voluntary basis prior to detection of drug use. Employees found to be using drugs will be subject to dismissal.

Federal Bureau of Investigation

The FBI initiated a drug testing program for its trainees in 1986. The program will extend to special agents and other critical positions on an incremental basis, similar to that for DEA. Collection and testing of specimens is combined with that agency's as well. The full policy for dealing with employees found to be using drugs has not been announced at this time.

DEPARTMENT OF THE TREASURY

Several of the agencies within the Department of the Treasury are planning to initiate drug testing within 1986. The first agency to do so was the Customs Service, which has a direct involvement with the apprehension of drug smugglers.

U.S. Customs Service

During the spring of 1986, the Customs Service initiated a drug testing program with its senior staff being the first to be tested. The collection and testing of specimens is being conducted under a contract. Personnel in critical positions, such as agents and chemists, will be included in the program on a random basis.

Secret Service Uniformed Division

Applicants and probationary officers of the Secret Service Uniformed Division have been tested for a variety of drugs since 1984. The testing is part of regular probationary physical examinations and is conducted by the District of Columbia Police and Fire Clinic.

GOVERNMENT SERVICES ADMINISTRATION

The Federal Protection and Safety Division of GSA has recently established policy and procedural guidelines for urine drug testing of all contract guards hired by GSA to guard buildings in the Washington, DC, area. This program includes preemployment, incident-related, and annual physical testing. Consideration is being given to extending the program to include GSA's employees.

CENTRAL INTELLIGENCE AGENCY

Early in 1986, the CIA initiated a trial drug testing program for all applicants.

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

NASA conducts drug tests on all astronaut applicant finalists at the Johnson Space Center. The primary focus of the testing is to detect the chronic use of several prescription drugs, as well as phencyclidine and amphetamines.

U.S. POSTAL SERVICE

Several offices of the Postal Service test job applicants for illegal drug use. A general policy is in place to test for drugs in employees whose performance deteriorates. Plans for testing under other circumstances are being reviewed in light of protests from the postal workers union.

NUCLEAR REGULATORY COMMISSION--NUCLEAR POWER PLANTS

While the Nuclear Regulatory Commission (NRC) has no current policy requiring drug testing, NRC licensees (nuclear power plant operators) have been encouraged, on a voluntary basis, to have mandatory drug testing for all personnel working in vital areas of the power plants. According to a recent NRC report, 90 percent of all nuclear plants have some form of drug testing, and the remaining ones are considering the implementation of programs in the near future.

Because of the sensitive nature of the work involved in a nuclear power plant, some operators have developed rather strict programs. All of those who currently have programs include preemployment tests for drugs. Almost all others test in probable cause situations. At least one company utilizes a controversial, anonymous "hot line" for tips on drug dealers and users. Accusations have been made that the hotline has been used to harass employees, although it has uncovered many drug dealers. Being caught using drugs usually results in termination. Many companies provide some level of counseling or assistance, although this may apply only on a voluntary basis prior to being detected.

PUBLIC UTILITIES--ELECTRIC AND GAS

Many nonnuclear power companies and gas companies have implemented drug testing programs. These usually involve preemployment testing and testing for probable cause. Evidence of growing concern about drug use among employees is supported by the number of arbitrations conducted on behalf of public utilities. Typically, a union will file a grievance on behalf of an employee who has been fired for testing positive for drug use following some incident. The argument usually questions whether the employee was "intoxicated" on the job, as opposed to use of the drug off duty. Some companies have resolved this issue by setting detection or confirmation levels that serve as "per se" evidence of recent use or that denote a violation of the rules.

LARGE INDUSTRIAL COMPANIES

A survey taken toward the end of 1984 suggested that nearly 30 percent of the Fortune 500 companies were conducting preemployment drug testing.

The number testing employees is unknown. It is known, however, that many large companies have drug testing programs, some of which include all those conditions discussed in the first chapter.

The most typical program would be similar to that described for public utilities, except that many large companies have extensive employee assistance programs (EAPs). Employees who have alcohol or drug problems can avail themselves of professional help in the EAP, usually on a completely confidential basis. They can be referred to the EAP by a supervisor, baaed on well-defined criteria that would include deteriorating job performance, repeated absenteeism, and injury. Sometimes the referral is made following observed behavior, but a growing number of companies use drug testing as a more objective measure of drug use. It is common for an employee who tests positive to be given an opportunity to enroll in the EAP and be referred to an appropriate counseling or treatment service. If employees refuse, they may then be subject to termination. An employee who fails to satisfactorily complete counseling or treatment or is detected using drugs a second time is usually fired.

It is important to note that many companies are now including tests for alcohol as part of their preemployment screens, in addition to the common practice of testing for alcohol in accident and other probable cause situations. Caution must be exercised in the interpretation of test results for alcohol in urine, since there is significant variability between blood and urine concentrations. It is strongly recommended that alcohol be included along with drug tests on individuals in treatment, since multiple substance abuse is not uncommon.

A few companies have implemented random testing similar to that in the military. They argue that it is the fairest and most democratic form of drug testing, because it applies equally to everybody. In order to be fair, the random selection must be truly random and not a guise for focusing on certain individuals or classes of employees. In at least one company division, the employees have endorsed the random program over a probable cause approach, because it eliminates the potential for supervisor harassment or being "out to get" someone in particular. Also, a strong possibility exists of "stigmatizing" individuals who are taken in for testing under a probable cause condition. This does not happen in random testing because everyone goes.

Whether a company uses random testing usually depends on the nature of the work and the nature of the drug problem. A program similar to that used by the Navy offers a strong deterrent to drug use but will still detect the user who will not stop using drugs. Many legal questions remain about random testing, however. Although random testing is not specifically precluded by Federal law, some courts and arbitrators have been holding private employers to constitutional standards, which ordinarily apply only to government actions. Therefore, it is very important to have any program, random or otherwise, meet the constitutional tests for fairness, reasonableness, and due process.

SMALL COMPANIES

Many small companies are initiating drug testing on preemployment as well as other conditions. Although large companies can usually obtain volume discounts for testing services, and thus lower per-specimen costs, the overall

cost of drug testing has been lowered due to the increase in the volume of testing. The small company can also obtain a more reasonable price for the smaller number of specimens submitted to testing. A problem the small company faces is its inability to maintain an EAP. Several available group programs can be covered under insurance or benefit plans, but it is not uncommon for such programs to be too expensive for a small company. For many of them, firing is the only alternative for employee drug use.

THE PRESIDENT'S DIRECTIVE FOR FEDERAL GOVERNMENT EMPLOYEES

On September 15, 1986, President Reagen issued an Executive Order which mandated efforts in all agencies to make the Federal workforce a model for eliminating drug abuse in the national workplace. This includes the use of urinalysis for drugs of abuse on selected employees of all agencies. The means of implementing these directives are being rapidly developed and a government-wide drug detection program is expected to be in place in 1987.

SUMMARY

Many Federal agencies and private companies are conducting drug tests on job applicants and employees. Although the reasons for testing and the circumstances under which testing is conducted vary considerably, the main intent of these programs is to provide a drug-free environment for other employees and a safe service to the public. The programs that have been most successful usually include a clear communication to all employees and applicants as to the nature of the drug program and the consequences of detected drug use. Also, successful programs usually afford employees some type of assistance and a second chance. Finally, it is essential for successful programs to provide a reasonable and fair approach that includes procedures for due process, that is, a line of review and appeal.

Choosing a Laboratory

Robert E. Willette, Ph.D.

A number of important considerations need to be reviewed when selecting a system for drug testing. The first major consideration is to decide if testing will be done at the place of business ("onsite") or by an outside laboratory. Both systems have advantages. The second and more important consideration is the choice of laboratory used for confirmatory testing and/or all drug testing.

TESTING ONSITE

Methods available to companies for onsite drug testing provide an effective and easy-to-use means of conducting urinalysis programs within the organizational structure. Some of the advantages include simplified chain of custody, control and a greater sense of confidentiality, and immediate results.

Chain of custody is a critical factor in a valid drug program. Confidence in the program is derived from knowing that the specimen tested actually belongs to the person who gave it. Onsite testing reduces the number of people who handle a specimen, which reduces the potential for mistakes.

Control and a sense of confidentiality may be important factors in some organizations. Testing at the place of business offers these advantages although a working relationship with a professional laboratory will, in fact, provide the same coverage.

Another advantage of onsite testing is that it provides immediate results of the drug tests, which may be important if action must be taken involving a member of the organization.

In most testing situations, it is essential that all specimens that give positive results in the onsite tests be confirmed by an alternate method, i.e., one of the chromatographic methods such as gas chromatography-mass spectrometry. An outside laboratory should be used for this procedure. In some situations, repeated testing of a sample from the original specimen, coupled with adequate operator training, instrument calibration, and quality control measures, may provide sufficient evidence of drug use when combined with other evidence, such as observed behavior or counselor interviews or admission in treatment programs. Some States have upheld onsite testing under these rigorous standards for prison populations while others have not.

One of the most frequently cited disadvantages of onsite testing is the possibility for taking action on the presumptive positive result before a confirmation is received. For example, the Department of Defense provides for temporary removal of individuals in critical positions who test positive; they must be reinstated with full privileges if the result is not confirmed. However, concern exists about the "tainting" of the person. It is recommended that the onsite screening result be kept as confidential as possible to minimize such exposure.

TESTING BY AN OUTSIDE LABORATORY

The most reliable method of testing depends on a confirmatory test of a positive result using an alternative method of testing. An outside laboratory should be used for this procedure.

Using an outside laboratory over onsite testing has advantages. Primarily, these advantages center on the professional capabilities of a laboratory that are not available to those who test only on the premises. Inherent in the decision to use an outside laboratory is the need to ensure accuracy by selecting a laboratory of the highest caliber.

If all specimens are sent to an outside laboratory, they are handled by a staff of trained professionals who perform the tests. And an outside laboratory can perform confirmatory tests immediately following a positive result using an alternative method of testing, which avoids delays. Carrying out all testing in the same laboratory minimizes the possibility of sample mishandling.

Additional benefits from using an outside laboratory often include laboratory staff who can serve as expert witnesses in legal and labor action and who can answer technical questions about the drug testing methods. The laboratory can also use its expertise to help an organization establish sound specimen collection and storage procedures.

CHOOSING THE BEST APPROACH

The advantages and disadvantages of onsite versus laboratory screening, as described, suggest some of the factors that must be considered in choosing the most appropriate arrangement for an organization. It is necessary to have a technically oriented staff person available to operate the onsite equipment. The testing area must be able to be secured from unauthorized entry. It must have a refrigerator and adequate air conditioning in order to maintain proper temperatures for storage and use of the reagents. The operator must be reliable and trusted for his or her coufidentiality, although it is possible to blind the operator from the identity of the specimen provider.

If these criteria can be met, the need for immediate or prompt results would be a major factor in choosing to screen onsite. A careful evaluation of the relative costs should be made. If the testing situation presents few positive results, the onsite operation can be quite cost-effective, since extensive specimen handling is eliminated. Only the positives need to be shipped to the laboratory for confirmation.

SELECTING A LABORATORY

Two major types of laboratories can be used. Large laboratories available nationally or regionally offer testing for drugs of abuse through a toxicology service, in addition to routine clinical testing for serum glucose levels, hematology, and blood enzyme levels. Smaller laboratories often specialize in drug testing and offer national service through the mail or courier services.

As yet, no official registry of approved or certified laboratories has been compiled. The National Institute on Drug Abuse is currently developing standards for proficiency testing and accreditation of laboratories engaged in drug testing. The standards will be available sometime in 1987, along with a registry of laboratories meeting them. In the meantime, it is essential to understand what to look for in selecting the best laboratory available. The following list of factors will help.

Factors to Consider

Information. Find out from Federal and State agencies if the laboratory has been licensed in any government programs and how the laboratory performed. Only California, New York, and Pennsylvania have proficiency testing programs for drug testing laboratories. Get recommendations from experts in the field of drug testing programs.

Inspection. Inspect the physical plant. Observe organization and procedures for processing specimens.

Standard operating procedures. Review the laboratory's manual for standard operating procedures. The manual should include a detailed description of every step for specimen handling and analytical methods. Each page should be dated and signed to show that it is continually updated as the laboratory modifies procedures. What is the laboratory doing to ensure that test results are properly reviewed and recorded?

Chain of custody. Examine documentation on chain-of-custody procedures from the time the specimens are collected until results are reported. Special handling procedures should be in effect for employee drug testing specimens. These specimens should be separated from routine clinical specimens before sealed containers are opened.

Who has access to stored specimens and why? How are they stored? Positive specimens should be stored frozen in a secure place, and there should be a way to identify everyone who has had access to them. The laboratory should also be able to track exactly where each specimen was from the time it entered the laboratory until it was stored.

How are records and actual testing data stored? Who has access to them? They should be filed in an easily retrievable manner.

Handling flawed specimens. An agreement needs to be made with a laboratory about testing a specimen if there has been a flaw or an error in the handling of the specimen. In most cases, it is best to obtain a new specimen that cannot be faulted at a later date. Some laboratories will help train personnel who collect specimens to avoid such errors.

Specimen identification. After a container has been inspected, it should be assigned a special identifying number or accession number. This is the method for tracking the bottle, the request form, and the test result. Some laboratories place several copies of the same preprinted number on the specimen bottle. These extras are used to identify test tubes containing the specimen or any other container. Bar-coded labels are now in use to further ensure that accession numbers are not misread or entered incorrectly.

Specimen integrity. Integrity of the specimen is maintained by labeling specimen and aliquot containers, as mentioned. To maintain chain of custody, the original specimen container should never leave the secured or limited-access part of the laboratory.

A completely new aliquot must be obtained from the original container when doing a confirmatory test. Matching the numbers is essential in order to avoid mixing up the specimens. Only one specimen container can be open at a time.

Checking staff credentials. The staff of the laboratory must, at the very least, meet State requirements. These vary from State to State. The labaratory should have an internal certification program for each staff position.

Minimum standards include the following:

- The laboratory director or manager should generally have an advanced degree in chemistry or toxicology, preferably in analytical chemistry, forensic analysis or forensic toxicology. This person should be certified or licensed by one of the appropriate boards or societies.

- The technical staff should have formal training as laboratory technicians or technologists, chemists, or biochemists, or they should have comparable on-the-job training and experience. Technicians should be certified by the laboratory and appropriate outside bodies. Laboratory certification covers each procedure the technician performs and should include performance on quality control samples.

- Find out how staff performance is monitored on a daily basis. Examine the laboratory's certification program and when each technician was last certified.

Quality assurance program. This is a primary requirement for any laboratory. It should be comprehensive in that it provides constant surveillance of all aspects of laboratory operations.

QA programs measure accuracy of performance in specimen accessioning, identification of aliquots, and test results, availability of current maintenance records for instrument, records of calibration schedules and general maintenance of good operating conditions; appropriate documentation and handling of chain of custody procedures; proper level of technical competence of the laboratory personnel; and generally attention to all practices which assure accurate laboratory results.

Quality control. This is a significant part of the QA program. QC is intended to ensure the accuracy of results by including samples with known

concentrations with every batch of specimens that is analyzed. These samples are “open,” or known to the operators, allowing them to evaluate the performance of each batch. In addition, it is strongly recommended that the laboratory include blind samples in its quality control program.

The laboratory should make these data available for inspection along with evidence of its performance on proficiency test samples run on a "bind” basis. These blind samples can be obtained from another laboratory or a proficiency testing service. Review the quality control records. How are these samples introduced? Are blind samples truly indistinguishable to the technicians from regular specimens?

Review of Results. One of the most critical steps in an acceptable testing process is the final review of all results and reports. The laboratory should have a senior chemist or the laboratory director certify that the report being sent to the client is accurate, whether the results are transmitted electronically, by phone or by mail.

Technical assistance and expert testimony. The laboratory should be able to provide access to a well-informed staff capable of offering sound advice about drug testing, selection of appropriate cutoff levels, and interpretation of results.

In the event of legal or labor action, the laboratory must be able to defend a drug test in a hearing or in court through expert testimony. An expert witness who can defend testing methods and the scientific validity of results usually has a doctoral degree or considerable experience in the field of drug testing.

Supplies. An important consideration in laboratory selection pertains to the materials the laboratory will supply. Most will provide all the specimen containers, request forms, evidence tape or sealers for the bottles, packaging materials (like plastic bags and boxes), and mailing or freight forms required for specimen collection. Some laboratories also include overnight courier service as part of the price.

Reporting results. It is important to understand and contract for specific turnaround time in the laboratory. Many laboratories provide results within 48 to 72 hours after specimen pickup. If confidential hard copies are needed immediately, some laboratories will set up an electronic means of transmitting results, other than by telephone. Telephoned reports should be avoided, since this method is least secure and most prone to mistakes. If urgency is not a factor, a mailed envelope, clearly marked confidential and addressed to the person authorized to receive the results, is adequate.

Equipment maintenance. Examine equipment thoroughly to determine operation condition. Current maintenance records should be available for each piece of equipment.

Proficiency testing. The laboratory should be participating in at least one proficiency testing program. Determine what it is, and review the results. Contact the agency providing proficiency testing and ask about the laboratory’s accuracy in the program.

LABORATORY EVALUATION CHECKLIST

A sample checklist for evaluating proposals from laboratories follows. The scoring is arbitrary and optional, but its use does provide a simple means of ranking various choices. An actual site visit should be made to the top-scoring laboratories to determine if they perform the work as they claim they do.

DRUG SCREENING--LABORATORY SELECTION

Laboratory **Final Score**

Quality Of Services (60 points)

Test methods (20 points) Score _____
(Consider sensitivity, established reliability)

Screening:

Confirmation:

Internal chain of custody (10 points) Score _____
(Consider if description is adequate, methods of identifying samples, recordkeeping)

Quality assurance program (10 points) Score _____
(Consider use of standards, internal blind QC, certification of standards

Turnaround times, reporting of results (5 points) Score _____
(Consider how results are reported, timeliness)

Specimen pickup, shipping (5 points) Score _____

Provision for frozen storage (5 points) Score _____

Supplies (5 points) Score _____
(Consider form design, labeling, security of bottles and kits, instructions for use)

Services Total Score_____

Personnel (30 points)

Laboratory director/manager (15 points) Score_____
(Consider who will provide expert testimony)

Management staff (10 points) Score_____

Technical staff (5 points) Score_____

Personnel Total Score _____

Experience (10 points)

Current clients (5 points) Score_____

Court/arbitration experience (5 points) Score_____

Experience Total Score_____

General Comments

Reviewer_____ **Date**_____

Proficiency Testing and Quality Control Programs

Robert E. Willette, Ph.D.

In a 1985 article, Drs. Hansen, Caudill, and Boone* of the Centers for Disease Control (CDC) proclaimed a "crisis in drug testing," based on the results of studies conducted by CDC on the reliability of drug testing laboratories during the period of 1973 through 1981. Under funding from NIDA, CDC conducted a nationwide proficiency testing program wherein 10 samples were submitted to participating laboratories every 3 months. These "open PT" samples were used as a measure of how well the laboratory could detect the absence or presence of specific drugs when they knew they were being tested. It is interesting to note that not all laboratories could maintain a passing score, which was set at 80 percent. The "crisis" proclamation was derived from the results obtained from a limited number of selected laboratories to which the same samples were submitted on a douhle-blind basis. Not all laboratories did as well when tested blind.

The article referred to studies conducted in the 1970s on laboratories providing drug screening services to federally funded drug treatment programs. Although most of these laboratories did not use the technology currently available, the study dramatizes the need for adequate external quality control over laboratory performance. Furthermore, it has focused attention on the general lack of such programs.

This chapter will describe existing proficiency programs and present some additional suggestions for monitoring laboratory performance.

DEPARTMENT OF DEFENSE

For more than 10 years, the U.S. Department of Defense has monitored the performance of drug testing laboratories operated by the military services and, more recently, laboratories providing drug testing to the military under contract. The program is conducted by the Armed Forces Institute of Pathology (AFIP)

At present, the program involves both open proficiency test samples and blind proficiency testing samples. Twenty-four samples, spiked with varying concentrations of the six drugs for which the military conducts urine tests, are sent each month to the nine military laboratories and current contract laboratories. These samples are analyzed and the quantitative results

*Hansen, H.J.; Caudill, S.P.; and Boone, J. Crisis in drug testing: Results of CDC blind study. JAMA 253:2382-2387, 1985.

reported to AFIP along with copies of the screening and confirmation test records. The results must fall within two standard deviations of the group mean in order for the laboratory to maintain satisfactory performance.

The blind program requires that AFIP send certified negative and spiked samples to several field units that submit urine specimens on a regular basis. Currently, 24 negative and 12 positive samples are submitted each week to the laboratories. The submitting units must transfer the contents to their normal bottles and submit the samples along with others using social security numbers supplied by AFIP. Since these are sent blind to the laboratories, copies of all results are sent to AFIP for decoding. If any discrepant results are received, an investigation into the cause is conducted. Laboratories must maintain a 90 percent score on positive samples and not generate any false positives. If the latter occurs, the laboratory is not allowed to report out any further results and must retest all positives that were obtained for a 2-week period prior to and following the occurrence of the false positive.

Although this level of external proficiency testing seems intense, the military drug testing laboratories process more than 200,000 specimens per month. In the last 3 years, no false positive result has been reported out on a quality control sample.

STATE PROGRAMS

The States of California, Pennsylvania, and New York conduct proficiency testing programs on certain drug testing laboratories in their jurisdictions. The California program is now limited to those laboratories conducting tests for State-approved methadone treatment programs. It is a blind program wherein several samples per month are submitted to the laboratories through selected treatment facilities. Pennsylvania sends four open PT samples per quarter to laboratories that perform drug testing on Pennsylvania urine specimens, whether in or out of State. The New York program is also open, with eight samples sent to every laboratory licensed to conduct such testing, whether in or out of State. Laboratories are tested this way each quarter unless their score is judged acceptable. Then, they are tested only twice a year.

COLLEGE OF AMERICAN PATHOLOGISTS

Since the late 1940s, the College of American Pathologists (CAP) has conducted interlaboratory comparisons designed to assess the state of the art in clinical laboratory practice. The program includes laboratory inspection, certification, and proficiency surveys. Starting in 1984, CAP initiated a survey for urine screening for drugs of abuse. It consists of sending to subscribing laboratories five urine samples containing five to six drugs each quarter. The laboratories analyze the samples and report the qualitative and quantitative (if they offer that service) results back to CAP for review. The laboratories are provided with an evaluation report.

Laboratories that also conduct analyses of blood samples can subscribe to the older toxicology surveys that include three or four samples of serum and one or two samples of urine containing various drugs in toxic concentrations.

AMERICAN ASSOCIATION OF BIOANALYSTS

The American Association of Bioanalysts has provided a variety of proficiency testing programs since 1949. A urine toxicology program became available in 1980. This survey provides subscribers with two urine samples per quarter, each containing 10 of the more commonly abused drugs or their metabolites.

DUO RESEARCH INC.

A consulting firm specializing in assessing drug testing programs, Duo Research Inc. provides a blind proficiency testing service to government and business. The service is similar to that described for the Department of Defense, in that positive and negative samples are sent to sites that collect and submit urine samples to a laboratory for drug testing. The number of samples received by subscribers is tailored to the particular level of testing being conducted. Monthly status and quarterly summary reports are provided. An investigation is made in the event of an incorrect result.

INDIVIDUAL PROGRAMS

Some laboratories conduct their own external as well as the mandatory internal quality control programs. Some laboratories with multiple locations may submit samples to each laboratory director to insert in a blind fashion into their routine testing. Others will provide customers with prepared samples that they can submit back to the laboratory in a blind manner. Although these are valuable adjuncts to other measures of performance, they are not as secure as those programs that provide an independent source of samples and interpretation.

AVAILABILITY OF REFERENCE MATERIALS

One of the difficulties in conducting quality control programs is the limited availability of suitable reference materials. Experiments with urine collected from individuals who are known to have taken a specific drug have shown a significant variability in sample stability. It has thus been favored to add known amounts of pure drugs and/or metabolites to a pool of drug-free urine. Collecting sufficient quantities of the latter also presents its difficulties. Some programs, such as that conducted by AFIP for the military, use a combination of human urine and a synthetic matrix. This can pose problems with assay interference and recognizability.

The most reliable proficiency testing and control samples are made from drug-free human urine where drugs and/or their metabolites have been added in concentrations consistent with normal detection ranges for the drugs in question. Also, as is the case with morphine, a mixture of parent drug and its conjugated metabolite must be added in order to simulate a real positive specimen. Proper samples for methadone contain at least two of its metabolites in order to be properly identified on thin-layer chromatographic analysis.

Few sources exist for some of the pure drug and metabolite standards. The National Institute on Drug Abuse has maintained a program for preparing such standards through a contract with the Research Triangle Institute (RTI), but these materials are available only on a limited basis for research purposes. Analytical quantities of pure drug standards can be obtained from Alltech-Applied Science, State College, PA; Supelco Co., Bellefonte, PA; and RTI, Research Triangle Park, NC. Larger quantities can be purchased directly from RTI.

To ensure the highest standards of drug testing, every government agency or private employer testing job applicants or employees must insist that the laboratory being used participate in at least one, but preferably several, of the proficiency testing programs described here. This should be a requirement written into the contract with the laboratory, and test results should be submitted to the customer as soon as they are received. Open proficiency tests can indicate the best that a laboratory can do when it knows it is being tested, but a blind program is most effective in consistently encouraging maximum laboratory performance.

Specimen Collection and Handling

Joseph E. Manno, Ph.D.

The urine drug test can be useful and reliable for determining drug use patterns only if it is performed and interpreted using appropriate procedures. The validity of the result of a urine drug test requires that consideration be given to the methods used for the collection, transportation, analysis, and interpretation of the results.

RESPONSIBILITY

Three different groups are generally associated with the urine drug test procedure. The collection site is responsible for collecting, labeling, boxing, and shipping samples, ensuring that collection and storage procedures have the proper documentation and security methods necessary. The collection site also must provide all staff with sufficient training to understand the collection process and the significance of laboratory results.

The courier is responsible for transporting samples to the laboratory and maintaining appropriate chain-of-custody records for ensuring that samples are not tampered with during transit. The laboratory is responsible for receiving samples from the courier and further guaranteeing that the samples were transported without tampering. The laboratory must also maintain records to assure that the integrity of the samples is maintained in the laboratory and that the results are correct. Although it is recognized that each collection site must develop collection and shipping procedures that meet its individual needs, the purpose of this discussion is to describe procedures that will fulfill the necessary criteria in order to guarantee optimum validity of drug screening results.

RANDOM SAMPLING

Random sampling is a process whereby urine samples are collected in a manner that the subject cannot predict when the specimen will be requested. Random sampling is most important when multiple samples will be collected from the same person over a period of time. Random sampling principles should also apply when single samples are collected for on-the-job drug testing programs. For several reasons, random sampling procedures are important to increase the overall "sensitivity" of the drug detection program. The laboratory procedures for performing the urine drug screen have defined sensitivity. Under most circumstances, intermittent drug use (i.e., taking drugs on less than a daily basis) can be detected within a range of 6 hours to 2

days or more after the drug was ingested. When individuals take drugs chronically (on a regular basis, either daily or more than once a day), the likelihood of detecting the drug in urine increases. However, it is possible for individuals to tailor their drug use to conform to the pattern of urine collection used. If they know a collection is forthcoming, they can discontinue use of the drug long enough prior to the urinalysis so the drug use will not be detected.

In substance abuse treatment centers, for example, if urines are collected regularly at specific times, the subject can start using drugs immediately after the collection and stop a day or two before the next scheduled collection. With a 2- or 3-day drug-free period before collection, a good possibility exists that the urine drug screen will be negative.

When urine drug tests are used as part of a preemployment program, if an individual is told to report for a preemployment physical at a prearranged tie, drug use could be discontinued prior to the appointment, thereby increasing the chance for a negative screen. With the proliferation of Laboratories, subjects may have enough time prior to a scheduled urine collection to have their urine analyzed for the presence of drugs. If the urine was found to be positive, they could then make an excuse for not keeping the appointment. The most effective random sampling schedule for urine collection is to contact subjects and require that they come immediately for urine collection.

In substance abuse treatment facilities, random sampling increases the effectiveness of the drug testing process, because it removes the regularity needed for patients to schedule their drug use in order to adjust drug taking so the screen is negative. Subjects are always in the position of not knowing when the next screen will be taken; therefore, they can never safely take the drug.

For preemployment drug testing, every attempt should be made to provide only several hours' notification before the subject must report for the test. Ideally, prospective employees can be given a 1 or 2 week "time window" during which they will report for the test. Then, notification can be given several hours in advance of the test. As a matter of practice, however, this may be very difficult to achieve in a preemployment situation.

Random sampling for on-the-job testing is more easily accomplished, since the employees are readily available. The determination of testing days and departments should be kept confidential. Employees who are absent on the day of testing should be tested at a later, unscheduled date.

When a program of "probable cause" or "suspicion" is in force rather than random sampling, samples are taken when a supervisor determines that the behavior of the individual is cause for testing. Obviously, in such a case, the specimen should be taken with as little forewarning as possible.

COLLECTION

The collection process is quite important, and several factors must be considered in developing a suitable method. The validity of the results of a urine drug test is dependent on the integrity of the specimen. The urine container obviously must be clean, unbreakable, and leakproof. Since the

analysis procedure used by laboratories has defined limitations, anythiug that might cause the urine to be outside these limitations would produce an invalid test. The way to achieve many of these limitations is common knowledge to some individuals who undergo urinalysis. There are many ways for a urine specimen to be invalidated if the individual has the opportunity.

- Individuals have reportedly placed various chemical substances under their fingernails and released them into the urine sample to affect the subsequent analysis.

- Placing a pinhole in the bottom of the urine container would result in a leak that would not be detected at the collection site. During shipping, however, most or all of the urine could leak out.

- Ordinary table salt, detergent, or other commonly available household chemicals can destroy the drugs or affect the assay in such a manner as to generate a false negative analysis. Frequently, soap dispensers or cleansers in toilet areas offer the opportunity to add effective adulterants to the sample.

- Use of a fluid-filled bulb placed under the arm, with a tube leading to the genital area, is another method. The subject can squeeze the bulb and release water or other substance that would dilute or contaminate his/her own urine.

- The subject can obtain urine from friends not using drugs or save his/her own urine from drug-free periods. This urine can be placed in the container during the collection period.

- The subject can scoop water from the commode into the collection container and dilute the urine.

It is important that specimen collection be directly witnessed if at all possible by a reliable individual to prevent this sort of intentional adulteration. While direct-observation collections provide the greatest credibility to a drug deterrent program, the procedure can be embarrassing to both parties. Where it is determined that privacy of the individual must take precedent over other considerations (and such was the determination in President Reagen's recent Executive Order for urine drug testing in the Federal workforce), there are means by which adulteration of samples can he minimized. The temperature of the specimen should be close to body temperature (37°C)* if the sample has not been diluted with water. This can be checked by thermometer. Collection facilities can be set up with no soap dispensers or cleaning agents available that can be used to adulterate the sample. The water in the toilet can be dyed or the toilet itself can be a chemical one, eliminating in both cases the availability of water for dilution. Such a facility should be considered in circumstances where specimens can not be witnessed as a matter of policy or where the possibility of the subject's bringing in something to contaminate or dilute the sample is unlikely. A patient undergoing a physical, for instance, may not be dressed and could not likely conceal anything to invalidate a sample by adulteration.

*Judson, B.A.; Himmelberger, D.U.; Goldstein, A. Measurement of urine temperature as an alternative to observed urination in a narcotic treatment program. Am J Drug Alcohol Abuse 6:197-205, 1979.

In most cases, the laboratory is capable of detecting the adulteration of urine specimens when water has been added by checking the specific gravity of the urine. A colored water solution or a urine to which a large amount of water had been added would have a lower than normal specific gravity. A pH check would indicate an attempt to acidify or alkalinize the specimen to invalidate screening assays. If adulteration is suspected, the laboratory should be notified and requested to make such a determination. The laboratory can also check on the validity of the sample by performing a creatinine analysis. Creatinine is normally present in a urine sample and will be detected by the test if the sample is urine. In addition, the concentration of creatinine can be used to determine if the sample has been diluted (by adulteration or by drinking excessive quantities of liquid).

Other means of influencing the outcome of a urinalysis that are more difficult to detect involve the ingestion of large quantities of water before providing a sample, which in effect dilutes the urine produced. Drinking large volumes of water or other liquid several hours prior to the urine collection could easily result in a tenfold dilution of urine. This dilution could lower the concentration of drug sufficiently so that it could not be detected by the laboratory analysis.

There is a widespread belief that drinking vinegar can produce negative urinalysis results. While it is theoretically possible that sufficient vinegar ingestion could alter urinary pH, it is highly unlikely that such a quantity could be drunk without toxic consequences. Even if pH were altered slightly, the effects on different drugs would be variable--the excretion rate of some might be increased slightly and for others it might be decreased.

SECURITY

The security of samples as well as empty cups, laboratory invoices, cup labels, and other packing or shipping material is critical. If subjects can obtain empty cups or other laboratory material, it becomes quite easy to substitute other urines for their own. Computer-printed labels should be attached to the cup, rather than to the top, to make it more difficult for subjects to switch samples.

The specimen donors should not generally be permitted to have any involvement in the collection, labeling, boxing, packing, or transporting of samples to the laboratory. It is important, however, that donors witness the sealing of the bottle and sign or initial the seal. Access to collected urines or any of the boxes, cups, tape, labels, or other laboratory materials should not be allowed.

After collection, urine specimens should be stored under locked storage conditions. If transport of the specimens is inordinately delayed, they should be kept refrigerated (4°C) if possible.

DOCUMENTATION

Accurate and complete records of all individuals involved in the urine collection, storage, and shipping procedures should be maintained. At least two major documents will be utilized for documentation procedures. These include a label attached to the urine specimen container and a separate invoice or other listing of the samples transmitted to the laboratory.

Specimen Label

The specimen label should be affixed to the urine container and not to the lid. This will prevent accidental or intentional switching of specimens and identifying labels.

The label should contain the following information:

- Name or other identification of the collection site or client
- Date and time the sample was collected
- Name or identification (social security numbers are frequently used) of the subject (subjects should initial the label and thereby acknowledge that the specimen is their urine)
- Name or identification of the individual who witnessed the urine collection (the witness' initials should also be on the label)
- Log number to link the specimen to the transmittal invoice, although the subject ID number from the label along with the site code number is generally sufficient for this purpose
- Approximate volume of urine collected

If possible, certain information on the labels, such as the name of the laboratory and/or the identification of the collection, should be preprinted. This will reduce the chances of subjects' switching labels or samples. AU writing on the labels should be in ink that will not run if it becomes wet from condensation of water or urine spillage. Clear plastic tape over the label provides an excellent mechanism for preserving the integrity of the information.

Invoice

A transmittal invoice that accompanies the urines will allow the laboratory to check the individual urines against the invoice to confirm that all the specimens collected actually reach the laboratory. The minimum information the transmittal invoice should contain is:

- Collection site or client name or identification number
- Subject name or identification code
- Accession number of the specimen (if used)
- Specimen collection date
- Desired tests to be run on the urine specimen (if are not preset)
- Name or identification number of the witness and/or persons responsible for collection, handling, storage, or packing of specimens at the collection site

PACKING AND SHIPPING

Sample identifications should be checked against the shipping invoice as they are placed in the shipping box. The staff member should ensure that the number of urines shipped and the tests desired correspond between the invoice and the urine cups.

The shipping container should be sealed at all openings with tape that cannot be removed. Additional security may be provided by the staff member's signing his or her name across the box and tape. In this manner, if the tape is removed, it will not be possible to reseal the box without detection. The laboratory should be supplied with a list of acceptable signatures.

Samples should be transported to the laboratory either by the courier or by a reliable staff member. If a bonded courier transports the samples, a record is kept which acts as proof of delivery for legal purposes. Courts have upheld shipment of such samples by U.S. mail, however. If the samples are delivered by a staff member, a receipt must be issued by the laboratory when the samples are received. Specimen donors should never be permitted to transport samples to the laboratory.

If the samples are delivered by courier, the invoice should be checked to make snre that the invoice accurately states the number of boxes sent. If the samples are delivered to the laboratory by a staff member, the staff member should request a hand receipt stating the number of boxes delivered.

If fewer than the number of boxes or samples stated on the invoice are received by the laboratory, or if a discrepancy is noted between the information on the container label and the invoice, a report of the situation discrepancy should be sent by the laboratory to the collection site.

Analytical Methodology

Richard L. Hawks, Ph.D.

The specific methods chosen by a laboratory will depend on a number of factors, including cost, workload (number of specimens), turnaround time, sensitivity required, and reliability. In nearly all applications of urine drug detection, a confirmation analysis is essential for all specimens screened positive. A different type of analytical methodology should be used for the confirmation analysis. Analytical methods used in most laboratories for the detection of drugs in body fluids can be classified into two main categories--immunoassays and chromatography--generally used respectively for screening and confirmation.

Examples of these two types of methodologies are presented, including sections describing how they are used for screening and confirmation purposes. A brief discussion of new trends in urine drug detection methods appears at the end of this chapter.

IMMUNOASSAYS

lmmunoassays are based on the principle of competition between labeled and unlabeled antigen (drug) for binding sites on a specific antibody. Antibodies are protein substances with sites on their surfaces to which specific drugs or drug metabolites will bind. These antibodies are formed by inoculating animals with appropriate immunogens. Two types of immunoassays are usually employed in urinalysis at this time--the radioimmunoassay (RIA) and the enzyme immunoassay (EIA). The difference between these types of immunoassays is mainly in the indicator that is used. The EIA utilizes an enzyme as an indicator in the assay (the label), while RIA uses a radioactive indicator. Because antihodies often cross-react with related drugs, and sometimes even with unrelated compounds, confirmation of positive immunoassay results with an independent procedure is imperative for definitive identification. The Abuscreen RIA manufactured by Roche Diagnostics in Nutley, NJ, is the RIA system most frequently used for drugs of abuse in this country (Abuscreen 1983). The EIA of most prevalent use in the United States is the EMIT manufactured by Syva Company in Palo Alto, CA.

Radioimmunoassay (RIA)

In radioimmunoassay testing (Chase et al. 1976; Ebert et al. 1981; Cook et al. 1982; Soares et al. 1982; Law et al. 1984; Cross et al. 1985). known amounts of radioactive-labeled drug are added to a urine sample with knowu amounts

of antibodies. The mixture is then allowed to incubate, during which time the labeled drug and unlabeled drug compete for binding sites on the antibody. After precipitation and centrifugation of antigen-antibody complexes, either the supernatant fluid or the precipitated antibody is transferred to a gamma counter (an instrument that determines the level of radioactivity of the sample). The presence or absence of the drug is indicated by the amount of radioactivity found, since this is proportional to the amount of antigen (labeled drug) bound to the antibody. If the supernatant is counted, a positive specimen is identified when the radioactive counts are equal to or greater than those of a positive control prepared in the same manner as that of the unknown urine. Conversely, when the precipitant (bound antigen) is counted, a positive specimen is identified when the radioactive counts are equal to or lower than those of a positive control prepared in the same manner as that of the unknown urine.

RIA can detect very small concentrations of drug with sensitivity ranges on the order of 1-5 nanograms per milliliter (ng/ml). The required sample volume is small and sample preparation is minimal. The use of automated pipetting and counting equipment allows for large-volume, multiple testing. Some of the disadvantages of this technique are associated with the use of radioactive substances and the high cost of reagents and instrumentation. Turnaround time is long--from 1 to 5 hours.

Enzyme Immunoassay (EIA)

Unlike RIA, EIA is a homogeneous enzyme immunoassay technique. That is, the antigen-antibody complex need not be separated by centrifugation. The most frequently used EIA method in this country is the EMIT system (Rogers et al. 1978; DeLaurentis et al. 1982; Law et al. 1982). In the EMIT assay, the label on the antigen is an enzyme (protein) that produces a chemical reaction for detection of drugs. This detection is based on the competition between unlabeled drug or drug metabolite and labeled drug or drug metabolite for binding sites on the antibody. Urine is mixed with a reagent containing glucose-g-phosphate (G-6-P) and antibodies to the drug, as well as a second reagent containing a drug derivative labeled with G-6-P dehydrogenase. The enzyme-labeled drug when bound to an antibody site is incapable of interacting with the substrate (G-6-P). If the enzyme-labeled drug does not bind to the antibody, then it is free to react with the substrate. The drug in the subject's urine competes for the limited number of antibody binding sites and thereby proportionally increases the total enzyme activity. The enzymatic activity is therefore directly related to the concentration of the drug present in the urine.

Some of the advantages of EMIT include (1) a short analysis time, (2) an easily measured nonradioactive endpoint that is simply measured, and (3) no necessary separation of bound and free fractions as in RIA. The EMIT, however, is generally less sensitive than RlA but still has moderate to good sensitivity and specificity (Allen and Stiles 1981). The enzyme/substrate interaction is somewhat more sensitive to temperature variation and ionic adulterants (such as salt). This procedure can be automated, allowing for multiple sample testing and reduced costs. Two systems are currently marketed--the EMIT-d.a.u. is designed for use in laboratories with large sample throughput; the EMIT-st is a portable system which can be used "on-site" and in situations where a small number of samples are analyzed in any given period.

CHROMATOGRAPHY METHODS

Chromatography is a method of analysis in which the various components in a biological specimen can be separated by a partitioning process. Chromatographic separations to resolve mixtures of drugs and metabolites require (1) a stationary (fixed) phase, which may be a solid or a liquid on an inert support having a large surface area, and (2) a mobile (moving) phase of liquid or gas. With a chromatographic method, substances are carried by the mobile phase through a column or across a plate, where the stationary phase interacts with the specimen to cause separation of the various components. After separation, a detection method distinguishes the components for identification and measurement.

Separation of the components of biological mixtures containing substances of various molecular types is based on the time spent by each component in each phase of the chromatographic system. Several different types of chromatographic techniques are used in laboratories for urine drug analysis. These various techniques offer different degrees of resolving power (the ability to separate one component from another) and often utilized in combination, depending on the drugs in question. While other chromatographic techniques are available, thin-layer chromatography (TLC), gas-liquid chromatography (GLC), and high-pressure liquid chromatography (HPLC) are the most commonly used ones. The combination of GLC with mass spectrometry (GC/MS) provides the most specific type of analytical tool currently used in urinalysis.

Extraction of biological samples is necessary for all chromatographic techniques for drugs other than alcohol. It is usually not required for the immunologic methods. Liquid-liquid extraction is the most commonly used method. This procedure involves the mixing of the sample in water with a water-insoluble organic solvent. If the drug of interest is more soluble in the organic phase, most of the drug is extracted from the water phase into the solvent. The solvent is then evaporated to dryness, and the residue is redissolved in a small amount of solvent and reserved for further testing. This is called direct extraction.

Some selectivity can be achieved by an appropriate choice of solvents and pH adjustment of the aqueous phase. Greater selectivity can also be obtained by a technique called multiple extraction (or back extraction) when necessary. Passing the drug back and forth from solvent to aqueous phase with pH adjustment tends to isolate it from nondrug or other interfering substances. Separation of classes of drugs can also be attained by this technique.

Liquid-solid extraction with resin or charcoal, and more recently other solid-phase extraction techniques, provide the necessary isolation of drug or drug metabolite from the biological sample and provide a relatively clean sample for analysis by GLC, HPLC, TLC, or related procedures.

Thin-Layer Chromatography (TLC)

Of the chromatographic techniques, TLC is one of the oldest methods but is still utilized as a practical technique for many large-scale multiple drug screening programs (Michaud and Jones 1980).

In TLC, an absorbent (stationary phase), such as silica gel, alumina, cellulose, or ion exchange resin, is uniformly applied to a glass plate or plastic film. Mixtures of known drug compounds (standards) or residues from an extraction of drugs from urine are applied as spots to prepared plates, which are then placed in a closed container with just enough solvent (mobiie phase) to wet the bottom of the plate. The solvent is allowed to flow across the stationary phase by capillary action, usually in an ascending fashion, allowing the substances to separate. The separated substances can then be identified by spraying the plate with reagents that produce characteristic color reactions. Drugs visualized in this way are identified on the basis of (a) reference values (ratio between the distance the mobile phase moves up the plate and the distance the compound moves from the point of application), (b) metabolic patterns (parent drug and characteristic metabolite), and (c) functional group analysis (chemical characteristics as defined by the color reaction with the spray reagent).

Advantages of using TLC are (1) low cost of equipment, (2) rapid analysis, and (3) ability to detect more than one drug or metabolite per analysis. Relatively small amounts of drugs can be detected, usually as low as of 0.5-1.0 micrograms per milliliter. The recent development of high-performance thin-layer chromatography (HPTLC) plates has enhanced the capability of thin-layer chromatography. In HPTLC, silica gel particle size and the thickness of the layer on the plate are reduced, allowing for the separation of drugs in much shorter distances. Increased sensitivity is also gained because the applied spots are smaller than those for regular TLC. A small spot in combination with the short migration distance results in high drug concentration at the migrated spot. The net effects are increased sensitivity for the detection of drugs and a shorter analysis time. References to TLC methods for a variety of drugs can be found in the last chapter of this monograph.

Some of the disadvantages of TLC are that it provides only fair specificity and sensitivity and results that are highly dependent on the technician's skill. It does require practice to recognize patterns of drugs and/or their metabolites by the visualized colored spots. These problems are minimized by commercial systems that attempt to standardize the elements of extraction, application, and visualization. One such system, manufactured by Analytical Systems, Laguna Hills, CA, is called the Toxi-Lab system. TLC also is a highly labor-intensive technique and sometimes requires extensive sample preparation.

Gas-Liquid Chromatography (GLC)

Gas-liquid chromatography (interchangeably referred to as gas chromatography or GC) is widely used in drug analysis as a confirmation method as well as a primary screening method under some conditions (ElSohly et al. 1984; Woodworth et al. 1984). It utilizes an inert gas, such as nitrogen or helium, as the moving phase to transport a vaporized sample of a drug through a glass column containing a stationary liquid phase. The drug is identified and quantified by a detector at the far end of the column.

The column's capability to separate and identify drugs is optimized by altering the types and amounts of a liquid (stationary phase) absorbed on solid-phase substances such as silica compounds. Typically, columns used in many GC methods are 3-6 feet in length and a few millimeters in diameter.

There has been great technical development in capillary columns, which offer an increase in separation power over the conventionally packed column. Capillary columns (0.2-0.7 millimeters in diameter and 5-100 meters in length) are made of fused silica and are available in several forms. With one type, the Liquid phase (organic compound) is present as a thin film on the capillary column walls. Bonded phases are widely used and offer several advantages over nonbonded phases, notably (1) on-column injections and (2) better stability and resistance to thermal shock. Capillary columns coupled with GC provide superior resolution of compounds, give greater sensitivity, and are generally recommended for applications in urine drug assays.

Either a conventional or a capillary column is placed inside an oven with precise temperature control; the sample is injected into a heated port and is carried through the column by the inert gas at a controlled flow rate. The column material has the ability to absorb substances as they are being moved from injector port to the detector. Different drug molecules tend to be differentially adsorbed by the liquid phase and the gas vapor phase. The equilibrium between these two phases as the drug passes through the column creates the characteristic column retention time for that drug. This retention time is the parameter of identification associated with GLC procedures.

Several types of detectors are available to provide the selectivity and sensitivity needed to properly detect and identify drugs of interest as they emerge from the column. Popular detectors are the electron capture detector (ECD), the flame ionization detector (FID), and the nitrogen-phosphorous detector (NPD). Each of these detectors has its own characteristics of sensitivity and specificity. The NPD detector is particularly suitable for nitrogen containing compounds such as phencyclidine or cocaine. The FID is of more general applicability, but is less sensitive than the NPD or the ECD detector.

Gas-Liquid chromatography is a sensitive technique, and small amounts of drugs can easily be detected and identified by determining their respective retention times as compared with known drug standards under optimum instrument conditions. Some of the limiting factors are (1) the slowness of analysis, since only a single sample can be processed at one time, (2) the expertise required in conducting the tests, and (3) the sample preparation time, since many drugs or their metabolites must be derivatized before they can become sufficiently volatile to move through the column. Additionally, GLC as well as all other chromatographic methods suffers from the deficiency that the retention time, which provides only a single parameter, cannot be used as an unequivocal identification in many cases. Certain drugs or conditions may require that other methods be used to provide rigorous proof of identity.

High-Performance Liquid Chromatography (HPLC)

This method is sometimes used in urine and blood analysis (ElSohly et al. 1983; Law et al. 1984a; Dye et al. 1984). It employs a column through which the drug passes while undergoing equilibration between two liquid phases, rather than a gas and liquid phase as in the case of GLC. Again, the characteristic of the drug molecule that is measured is the time it takes for the drug to traverse the column at a given solvent flow rate. Detectors are

ultraviolet, fluorescent, or electrochemical in nature. HPLC has the advantage that polar drugs requiring derivatization on GLC systems can be assayed directly on HPLC. Its disadvantages are similar to those of GLC, although specimen preparation may be simpler. Some laboratories take advantage of HPLC's chromatographic capabilities and the superior sensitivity of RIA methods by using RIA as the HPLC detector. In this case, fractions from the HPLC column are sequentially analyzed by RIA to provide a highly specific and sensitive system of analysis (Law et al. 1984a). This technique is useful in many forensic applications but generally is too cumbersome for routine urinalysis.

Gas Chromatography/Mass Spectrometry (GC/MS)

The analytical technique of gas chromatography/mass spectrometry (GC/MS) combines the efficient separating power of gas chromatography with the high sensitivity and specificity of mass spectrometric detection. GC/MS is generally considered to be the moat conclusive method of confirming the presence of a drug in urine. The major factors that have limited the use of GC/MS have been its comparatively high cost and complexity. Fortunately, GC/MS instrument manufacturers have recently introduced lower Priced systems that are easier to operate and this should result in significantly lower fees for GC/MS analyses.

In spite of the remarkable potential capabilities of GC/MS, it should not be assumed that the results of all drug confirmations performed using GC/MS are conclusive. There are many different modes of operating a GC/MS. It can be operated in the "full scan" mode which provides a complete mass spectrum for each component of the urine extract that passes through the gas chromatograph. Since a complete mass spectrum represents a "fingerprint" pattern that is unique for each drug, this mode of operation will give the most conclusive identification if there is a sufficiently high concentration of the drug to provide a good quality mass spectrum.

Alternatively, the GC/MS can be operated in the selected ion monitoring mode in which the mass spectrometer monitors the ion currents at only a few masses which are characteristic of a specific drug. This mode of operation affords far higher sensitivity, but provides a less specific pattern for identification. Other choices of modes of operation include the method by which the drug molecules are ionized. Electron impact (EI) ionization is the technique most widely used. Mass spectra obtained using EI ionization are typically quite complex and therefore very suitable for obtaining a "fingerprint" identification of a drug. The technique of chemical ionization (CI) is an alternative method of ionizing molecules for mass spectral analysis. CI mass spectra are typically less complex than EI mass spectra of the same drug, and therefore are less unique. However, because CI is often more sensitive than EI ionization, it can be used to detect and measure lower concentrations of the drug. Also, some methods of chemical ionization are relatively selective as to what compounds are ionized, as a result detection of a drug by CI mass spectrometry is often less subject to potential interferences from co-eluting components of the urine extract.

The choice of which modes of operation are to be used (full scan or selected ion monitoring, CI or EI ionization, etc.) depends on what drugs are to be detected, the minimum concentration of the drug that constitutes a positive

identification, and whether or not the concentration of the drug is to be quantitatively determined. The reliabitity of a GC/MS assay is also dependent on the skill and experience of the operator, as well as on the method used for extraction of the drug(s) from the urine and for preparation of the extract for injection into the GC/MS. An extensive knowledge of GC/MS technology is often needed to adequately evaluate the reliability of a specific assay, but if appropriate methods are used by well-trained analysts, a GC/MS analysis will ensure that a suspected drug is identified properly, and conversely, that no one will be falsely accused of drug use.

SCREENING PROCEDURES

The principle of screening by immunoassay, a highly sensitive technique, is to minimize the possibility of false negatives. By selecting highly sensitive techniques for broad classes of drugs, however, absolute specificity is lessened such that some false-positive results may be produced from cross-reacting substances having a similar chemical structure present in the specimen. For this reason, positive results from the screening procedure should be considered only presumptive; they must be confirmed by a second and distinctly different analytical technique.

Alternatively, thin-layer chromatography is often used as an initial screen when the ability to screen inexpensively for a large number of drugs is more important than the degree of sensitivity. Due to the level of subjectivity involved in the interpretation of TLC assays, however, it is important to confirm any presumptive positives with a highly specific method such as GC/MS.

SENSITIVITY AND ASSAY "CUTOFF"

The ability of any assay to detect low levels of drugs has an inherent limit. The concentration of drug in the urine sample below which the assay can no longer be considered reliable is the "sensitivity" limit. It is sometimes called the "detection limit" and is expressed as a concentration of the analyte in the specimen. The "cutoff" point is the concentration limit that will actually be used to assay samples. It is a value serving as an administrative breakpoint for labeling a urine result positive or negative. Manufacturers of commercial urine screening systems set cutoff limits to their assays well above the sensitivity limits of the assay to minimize the possibility of a sample that is truly negative giving a (fake) positive result. For example, although most immunoassays for detection of marijuana are sufficiently sensitive to detect drug metabolites at levels below 20 ng/ml, the assays are usually set for cutoff levels of 20 to 100 ng/ml. This decreases the possibility of a false positive that could result from operating the assay too close to its level of sensitivity.

The cutoff levels selected should be reasonable concentrations reflecting realistic urinary elimination of drugs. In immunoassay screening procedures, a cutoff standard is selected along with control standards at other concentrations. Any sample that contains the drug of interest at concentration levels equal to or greater than the designated cutoff is reported positive, and any sample that is less than the cutoff level is reported negative. Setting screening cutoffs too low would allow for longer detection

time after drug administration, but the results might be difficult to confirm reliably. If the confirmatory procedures are not sensitive enough, the screened positive may not be confirmed and the result would appear as a false positive.

The distinction between an unconfirmed positive and a "true" false positive is sometimes confusing. A sample determined positive by one method and negative by a confirmation (different) method could be a situation where a false result occurred in the first analysis and the drug was not in fact present, or, alternatively, the drug might in fact be present but was not detected in the second assay due to differences in the sensitivity of the two assays. On the other hand, setting high cutoff levels for the screening procedures will generate false negatives because drugs may be present in significant concentration but below the designated cutoff and would therefore be reported negative. Each urine sample must undergo a separate immunoassay for each of the selected drug groups. Depending on the laboratory and the technique employed, cutoff levels may differ. Therefore, negative screening reports should contain a statement with reference to established cutoff levels only, without providing the specific numbers leading to the negative evaluation. Urine specimens testing negative do not require a confirmatory test.

ASSAY SPECIFICITY

Specificity or selectivity of an assay method refers to the ability of the assay to identify a single chemical component in a mixture of chemicals and biological materials. This characteristic is a function of one or all of the processes of isolation, separation, and detection of a particular product in a biological matrix. A highly selective detector on an HPLC or GC can compensate for a complex mixture, while the same compensation may be achieved by an unusually efficient separation technique such as capillary column chromatography.

The most specific types of assay methods optimize all these factors. GC/MS, for instance, with a capillary column permits highly efficient separation of components on the column, followed by extremely selective detection in the mass spectrometer. It therefore achieves the most specific results of all assay methods.

CONFIRMATION PROCEDURE

The principle of confirmation procedures is to use a highly specific and alternate chemical technique to ensure that false-positive results do not occur at the selected or established cutoff levels. For most assays, the sensitivities for the confirmation procedures should be lower than the cutoff of the screening procedures used, so that the number of false negatives or positives due to concentration differences is minimized. In some cases (e.g., cannabinoids), the confirmation cutoff is generally set at a much lower level than the screening cutoff, because the immunoassay reacts additively with several metabolites from THC and the more specific confirmation methods are directed at only one. A selective liquid-liquid or liquid-solid extraction procedure prior to the confirmatory test is used to isolate the drug and/or metabolite from the urine. The purified extraction product may require further treatment (e.g., derivatization) before it is analyzed.

Prior to extraction, a known amount of an internal standard is added to each standard control and to each sample to ensure extraction integrity and to serve as a basis for quantifying the drug in question. The internal standard is a chemical compound having chemical and physical properties similar to the drug being tested. A distinct advantage for GC/MS is the ability to use a deuterium-labeled internal standard. In this case, the internal standard is virtually identical to the drug being tested, but it can be measured separately due to the different mass. A calibration standard containing the drug at the cutoff level is included in each analysis, as are blanks and positive and negative controls. (The quantitative analytical data are compared with calibration curves for the analyte with a known quantity of the internal standard.) AU quality control materials being run should give results within acceptable deviations from the true mean (generally ±2 standard deviations).

RECOMMENDED ANALYTICAL APPROACH TO DRUG TESTING

Because of the potential impact of the results of a urinalysis on an individual, only the most rigorous and conclusive procedures should be used. It is essential to incorporate both a screening and a confirmation step in any urine drug detection program where the consequences of such an analysis will he the basis of actions taken against the individual who supplied the sample. ln this regard, the confirmation techniques chosen by the laboratory should provide the most accurate and unequivocal results possible. While confirmation techniques other than GC/MS may be adequate for some drugs of abuse, GC/MS is generally accepted as a rigorous confirmation technique for all drugs, since it provides the best level of confidence in the result. Put another way, the appropriate use of GC/MS as a confirmation technique is least likely to become a topic of debate between expert witnesses at a legal proceeding. Major Federal drug screening programs, such as the one used by the Department of Defense, mandate GC/MS as the confirmatory method for all drugs.

Thus, an effective analytical system for the detection of drugs of abuse in urine should consist of (1) a sensitive, drug-class-selective technique such as EMIT or RIA, employed as the initial screening process to identify negative specimens and to select presumptive positive specimens, and (2) a highly specific technique such as GC/MS, used for confirmation of the presumptive positive results.

NEW DIRECTIONS IN URINE DRUG SCREENING

Because of the tremendous interest in drug screening today, efforts are being made in many areas to develop new techniques that would be applicable to this question. Some of these efforts are directed at developing new analytical systems that will be simpler and perhaps less expensive than conventional systems for drug screening or confirmation. In some cases, the goal is portability, such as in efforts to produce dipstick-type assays. Different biological media are being explored as alternatives to urine. Efforts are also being directed at developing noninvasive means of diagnosis, such as equipment to determine drug effects in the individual based on certain electrical outputs from the brain. Other attempts are aimed at developing methods or devices to better analyze impaired performance as an indicator of a drug effect, a phenomenon not measured by urinalysis.

Developments in screening systems are underway in the area of immunoassays. Because fluorescent systems have very high sensitivity and do not require the use of radiolabeled material as does RIA, fluorescent labels have become an attractive idea. Problems with background fluorescence have prevented fluorescence spectrometry from becoming highly useful in drug analysis in spite of the high sensitivity, but in recent years, systems have been developed that combine highly specific separation systems, based on antibodies with fluorescent detectors, to produce highly sensitive and specific fluorescent immunoassays. One example is the widely used TDx System manufactured by Abbott Laboratories. Abbott has recently marketed a new system called the TDx Abuse Drug Assay, which is designed to detect classes of drugs of abuse. It is to be expected that other systems based on immunoassay and fluorescent labels will be forthcoming in the near future.

Other research groups are investigating various ways to incorporate immunoassay specificity into systems that would give rapid readouts of presumptive drug presence based on a dipstick analysis of a urine, blood, or saliva specimen.

Work continues in the area of mass spectrometry to make this highly specific method more economical. The specificity and sensitivity of GC/MS are already sufficient for most applications of drug screening. A disadvantage in GC/MS analyses is the time required for the drug to traverse the chromatography column and for the analyst to prepare the sample for injection into the instrument, although ways to reduce this time element are being explored with an eye to using GC/MS as a screening as well as confirmation tool.

A liquid chromatograph can be connected to a mass spectrometer (LC/MS) in much the same way as the gas chromatograph is connected in GC/MS. As discussed in the previous section on HPLC, derivatization is usually not necessary and sample workup time is greatly decreased. While this type of instrumentation is not generally in use now in urine drug assays, it may have applicability in situations where confirmation analyses without derivatization are desirable.

Another type of new MS technique that may have application to urinalysis is called tandem mass spectrometry (MS/MS); this is particularly attractive because it can often eliminate the need for a chromatography column. It also offers the possibility of increased sensitivity and specificity over that of conventional GC/MS. An extremely sensitive assay for THC in rabbit plasma using this technique has been reported (Harvey et al. 1982). MS/MS is a technique that couples two mass spectrometers together, so that one acts as the sample cleanup system and the second as the ultimate analyzer. This approach theoretically would allow a relatively crude extract to be introduced directly into the first MS, eliminating the time-consuming chromatography step, while at the same time providing increased sensitivity, which may be necessary when drugs such as LSD and some fentanyl derivatives which are extensively metabolized and appear in extremely low concentrations in urine become the subject of urine screens.

OTHER BIOLOGICAL SAMPLES

Other types of biological samples, including blood, hair, and saliva, have been proposed as alternatives to urine for drug screening. Although studies on

analysis of blood for drugs have been extensive and such tests potentially provide a more specific indication of drug impairment, blood analysis generally requires more sophisticated techniques of analysis than urine. It also is considered a more invasive sample to obtain, and requires trained personnel to do so. Saliva is perhaps the most easily obtained sample. It has been established that many drugs can be detected in saliva (Caddy 1984). THC, for instance, is sequestered in the mucous membranes and can be detected (Norton and Garriott 1983; Gross et al. 1985; Cook 1986) for several hours after marijuana is smoked. Saliva testing is not generally used, because the methods of analysis are frequently more difficult than for urine. Further, the time period during which drugs can be detected in saliva after use is usually only a few hours, often paralleling plasma levels.

The analysis of hair has also been proposed as a way of detecting past drug use (Baumgartner et al. 1981, 1982). It has been shown that certain drugs can be detected in hair samples by means of sensitive RIA or GC/MS techniques, but the methods have not yet been sufficiently validated in clinical studies in comparison with urinalysis to adequately assess their suitability for general drug screening (Puschel et al. 1983). This type of screen also suffers from the fact that the extremely small sample (20-30 hairs) is frequently consumed in the screening assay, leaving insufficient material for confirmation by any but the most sensitive GC/MS techniques.

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Accuracy in Urinalysis

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Accuracy is the absolutely essential ingredient of laboratory analysis. The public perception of scientific measurements is that they are indisputable. If a laboratory reports the presence of a quantity of drug in a specimen, this ruling is judged to be correct, regardless of protestations to the contrary by the subject. Users of laboratory tests assume the results are reliably accurate; otherwise, the cost of these tests is wasted. But what is "accuracy," and how can it be estimated?

Accuracy can be viewed as the closeness with which test results agree with the "true" quantity of a drug in urine. In an unknown urine specimen, the "true" result, the center of the proverbial bull's eye, obviously is not known. Judging whether a specific test result is accurate can be done only by evaluating a laboratory's quality assurance program (Annino 1978).

Quality assurance (QA) is a term used, to represent those practices carried out to assure that specified quality goals are achieved (Eilers 1975). Once the quality goals have been defined, quality control (QC) procedures can be designed to monitor performance parameters. Thus, it is important that the testing laboratory and the user of the test results clearly understand the goal of the testing program.

Urine drug testing programs can demonstrate the presence of certain drugs or drug metabolites in urine. These results alone cannot be used to determine dosage or the time of drug administration or the extent of any drug effects in the subject, but urine drug testing programs can indicate drug use. They may also provide presumptive evidence that certain behavioral changes or decrements in performance observed in the subject may be associated with drug use. Thus, the goal of urine drug testing may be stated as the reliable demonstration of the presence, or absence, of specified drugs or metabolites in the specimen--that is, the production of a valid positive or negative result.

FACTORS CONTRIBUTING TO ACCURACY

Generally, when large numbers of specimens are processed by a laboratory, screening tests are used to separate the greater number of negative specimens from those producing positive results. Since screening tests are subject to interferences, all specimens that test positive must be confirmed by an independent, more specific procedure. If a relatively high concentration of drug is present in the specimen, screening and confirmatory

tests are more reliable. As the drug concentration approaches the limit of the test to detect a drug, more uncertainty is associated with the test result. This involves another aspect of lesting procedures known as "sensitivity."

Sensitivity is the lowest concentration of a drug that can he reliably detected by a particular test procedure. The key word here is "reliable." A test may be considered very sensitive when measuring a pure drug, but the test's reliability may be poor when the drug is in a complex matrix such as urine.

Most laboratory instruments are devices that measure drug concentrations by means of a detector. The detector may be a gamma counter in radioimmunoassay, an optical device in fluorescence or enzyme immunoassay techniques, or more complex in nature for some chromatographic methods. The detector, in turn, generates an electronic signal, which drives a printer or recorder to permit the analyst to visualize the intensity of the detector response and thereby estimate the amount of drug present. All detectors produce a certain amount of background "noise." When tests are run near the limit of sensitivity to a drug, it is increasingly difficult to discriminate between the noise and a true signal in response to a small amount of drug.

In order to compensate for sensitivity problems, and possibly minimize the incidence of positive results from passive drug exposure, frequently a cutoff concentration is selected. Above this level, a test is considered positive, and below it is considered negative. A disadvantage of establishing cutoff concentrations is that some individuals with actual drug present in the urine, albeit at low concentrations, will be deemed negative. These false-negative tests are generally tolerable in order to avoid false-positive results due to sensitivity variability.

Cutoff levels, when adopted, require consideration of another parameter of drug testing methods known as precision. Precision is the degree of agreement between repeated measurements. Good precision of a testing method increases confidence that the test can discriminate between drug concentrations above or below the cutoff level. Precision can be measured by the standard deviation (s) or coefficient of variation (c.v.) of a method. These are indicators of random error.

Consider the example in table 1. A cutoff level of 100 ng/ml has been set for a test for a metabolite of THC. That is, a test showing greater than 100 ng/ml of this metabolite will be called positive; below 100 ng/ml the test is negative. In order to determine the precision of this test, a specimen is divided into 10 portions and each portion is assayed.

One result, test number 6, appears to be unusually different from the rest. This result can be examined statistically to estimate the probability that it is an extreme value and can be rejected. When this is done, it is found that the probability that result number 6 is an "outlier" is greater than 995 times in 1,000. An outlier is a test result far removed from most of the other test results on the same specimen. Thus, it can be rejected.

Note that the "true" value is not known, but repetitive testing can result in a valid estimate of the true value. When the outlier is included, the mean is 102.1 ng/ml (interpreted as positive), but when the outlier is excluded, the mean is 99 ng/ml (interpreted as negative).

Table I

Test No.	Result (ng/ml)	Result (ng/ml)
1-	98	98
2-	101	101
3-	95	95
4-	99	99
5-	103	103
6-	(130)	
7-	100	100
8-	97	97
9-	96	96
10-	102	102
Mean (x)	102.1	99.0
Standard deviation (s)	±10.14	±2.74
Coefficient of variation (c.v.)	9.93 %	2.77 %

This example is chosen to illustrate two aspects of analytical testing:

1. The necessity for excellent precision to discriminate between positive and negative when the concentration of the analyte is near the cutoff (or limits of sensitivity) of the test

2. The troublesome problem of outliers

At times, a careful review of procedures used can explain why the unusual result occurred, but frequently no obvious explanation can be found. When repetitive analyses are done, outliers can he recognized and tested statistically. Unfortunately, if a single test is done on an unknown specimen, it is not possible to know whether the result obtained is an outlier or an estimate of the true value within the standard deviation of the test. It has been reported that, in intralaboratory testing, 5 percent to 15 percent of the results may be statistical outliers (Horwitz 1982). Only repetitive analyses of the same specimen can identify these discrepant results.

Tests for urinalysis should be precise and accurate and generally accepted by the scientific eommunity. Such tests are of two types, screening and confirmatory. Screening tests must reliably identify negative specimens, although it is recognized that some weakly positive specimens will be interpreted as negative due to sensitivity or precision limitations of screening procedures or due to negative outliers. Since negative results by screening tests are generally of little concern, confirmation of these is not necessary.

Positive results of screening, on the other hand, should be repeated. If still positive, the test must be confirmed by independent procedures of greater specificity and precision. This is to better characterize the drug, confirm its presence at concentrations above any cutoff level, and identify outliers. It is also important to carefully document and follow specific criteria that defii a positive versus a negative sample with any particular analytical methodology.

Implementing these procedures, monitoring their performance, and identifying and correcting defects in the testing procedure require continuous attention to a quality assurance program.

QUALITY ASSURANCE

A quality assurance program includes all of the practices carried out by the testing laboratory to assure that the goal of reliably identifying urine specimens containing defiied quantities of specified drugs or drug metabolites is achieved (Tietz 1986). All of the parameters of drug testing (such as accuracy, precision, detection of outliers, etc.) are monitored by quality control procedures. QA involves all aspects of the testing laboratory. Specimen acquisition, processing, testing, and reporting of test results must all be as error free as possible in order to achieve the goals of urine drug testing.

Dedication to quality testing must be of paramount importance to the laboratory management. Not only must laboratory directors and supervisors be committed to these goals, but administrative support is essential to provide a budgetary basis for space, equipment, staff, and all other resources necessary for quality testing. The cost involved in maintaining QA must be recognized at the outset.

PERSONNEL

The qualifications of personnel are important in achieving quality results in urine drug testing. Different tasks require different degrees of training and experience; all require attention to details and a disciplined approach to carrying out procedures. Graduation from an accredited school of medical technology with certification in clinical chemistry qualifies most individuals to operate automated instruments, carry out laboratory recordkeeping procedures, and implement quality control, all under supervision. Analysts operating chromatographic instruments require additional training and experience in these techniques. An individual holding a baccalaureate degree in chemistry from an accredited university should understand the necessary basic theory of instrumental analysis to permit the recognition of aberrant results and the ability to “troubleshoot” an instrument that does not meet specifications.

Supervisors and laboratory directors must possess these qualifications as a minimum. In addition, experience and advanced training in analytical methods and pharmacology or toxicology are required to understand the strengths and weaknesses of the methods used to identify drugs, discriminate between real knowledge of drugs and their effects as opposed to conjecture, and interpret the test results reported by the laboratory.

Certification in clinical chemistry (by the American Board of Clinical Chemistry) or forensic toxicology (by the American Board of Forensic Toxicology) is highly desirable. These individuals must be sufficiently skilled and knowledgeable to defend and interpret the laboratory results in court, if necessary (Forney 1978).

SPECIMEN ACQUISITION

Drug testing laboratories are involved only rarely in actually obtaining the specimen being examined. Obviously, collecting a valid, uncontaminated specimen uniquely identified as from a specific individual is a key issue in urine drug testing. Details relating to the specifics of specimen acquisition are discussed in a previous chapter.

Once the specimen is collected, it is marked in a unique way traceable to the individual giving the specimen. A name is normally not sufficient. Rather, a unique number such as a social security number, employee number, etc., is preferred. A record of the name, unique identifying number, time and date of collection, and the identity of the individual receiving the specimen is made. The specimens are packaged in a secure manner, marked "Urine Drug Screen" (or flagged in a less obvious manner), and sent promptly to the laboratory by courier, U.S. mail, or other secure means.

Upon receipt by the laboratory, the title "Urine Drug Screen" alerts the receiving clerk to forward the unopened package to the individual assigned to receive this type of specimen. A log is kept, listing name of the subject, unique identifying number, laboratory accession number, time and date received, condition of the specimen, and name of the individual carrying out the task of logging in the specimen. The laboratory accession number is firmly affixed to the specimen container, and it is stored securely at 4°C until analyzed.

This process, termed "chain of custody," must be followed in handling specimens of legal significance. Records of all individuals, both in and outside the laboratory, having access to the specimen must be kept, and these records documented and preserved for future reference.

Errors can occur in this phase of specimen processing. Transcription errors, misspelling, and even confusion of specimens can occur. Quality control can be exercised by proper training and motivation of personnel involved and by computer tracking of the specimen, together with computer printing of labels and accession numbers. Further, artificially prepared specimens with known errors can he introduced into the system. Technicians receiving the specimens are made aware of this check but are not informed as to the frequency of the checks. In a similar fashion, other steps in specimen processing can be monitored.

CONTROL OF ANALYTICAL VARIABLES

A number of factors relating to the environment, water quality, power sources, and other general aspects of a laboratory may affect analytical testing and must he monitored. Measuring devices of all types--balances, pipets, thermometers--must be monitored frequently to determine if they

meet prescribed tolerances and reliably indicate true values. lnstruments of all types must be checked routinely to maintain calibration and confirm that they are performing according to specifications. All of these monitoring procedures must be documented and reviewed regularly. When properly implemented, review processes not only ensure that monitoring procedures are conducted but identify emerging problems before they become critical (Blanke 1978).

Analytical Methodology

The choice of a valid analytical procedure is an obvious and important factor in achieving results of high quality. The specificity, sensitivity, and reproducibility of the method must be known in order to achieve the quality goats selected previously. Facility in the application of the method to real samples by routine personnel must be demonstrated. The method must be described in detail in a procedure manual. Any deviations from the method must he approved by an authorized supervisor and documented. Improvement in methodology is a continuous process and should be encouraged, provided all improvements are validated. New and novel procedures should not be used in routine testing until accepted by the scientific community.

Standards

The standards by which a method is calibrated must be selected with care. Generally, drug standards can be obtained from Supelco Inc., Bellefonte, PA; Alltech-Applied Science, State College, PA; Sigma Chemical Co., St. Louis, MO, or the United States Pharmacopeia, Rockville, MD. Other sources such as pharmaceutical companies or chemical suppliers may be used. In all cases, efforts to assess the purity of the standard by chromatography or by measuring a physical constant should be carried out frequently, since some drugs degrade with time. Accounting for the degree of hydration of crystalline substances can be particularly troublesome. Obviously, any contaminant, whether water or another substance, is weighed out as the drug when a standard solution is prepared. Unless the magnitude of the contamination is known and corrected for, an error is easily introduced (Blanke 1978).

Controls

An important aspect of any quality control procedure is the control material or control specimen. This is a urine specimen containing a known quantity of the drug(s) being tested that is run along with the subject's specimen. Controls can be purchased commercially or prepared by the laboratory. Ideally, they are specimens containing the drug(s) in a physiological state, i.e., present in the same matrix and environment as it would be in a patient. Metabolites, conjugated forms of drugs or metabolites, interferences, endogenous substances, all are present in the control. In practice, control material can be collected by pooling excess specimens. The well-mixed material is aliquoted into small volumes and frozen until used. By repetitive assays, a mean concentration of the analyte is measured with a calculated standard deviation. Thereafter, for an assay procedure to be "in control," the control result must agree with the known mean value within ±2 standard deviations. A result outside of this range is deemed "out of control," and no

results are reported until the cause of the discrepancy is identified and the assay again meets acceptable criteria.

Control material for screening procedures should contain analytes at or near the cutoff or sensitivity limit of the test. A negative control with the analyte below the cutoff and a positive control with the analyte slightly elevated above the cutoff will permit the analyst to determine when the teat is performing properly.

Control results are documented and recorded on a chart or in a computer program such that trends in control results may be visualized to enable a trained analyst to discover problems in an assay before they become critical In addition, users of laboratory results should inspect these control charts regularly and make sure that this documentation is retained if required in cases that may be challenged in court.

EXTERNAL QUALITY CONTROL PROCEDURES

The QC procedures described earlier are designed to detect changes in performance during routine operations, as compared with the careful setup procedures used when the method was initiated. However, if the method was not set up accurately (e.g., impure standard, miscalibration of a key measuring device, etc.), or if other methods perform more acceptably, internal QA procedures do not suffice.

Extemal QC procedures permit a laboratory to be compared with other laboratories. External QC may be of two types: open proficiency testing (PT) or blind proficiency testing. These two types of QC procedures have been reviewed in a series of papers that appeared in the May/June 1977 issue of the Journal of Analytical Toxicology.

In open PT, a sponsoring group prepares a large quantity of control material and sends a portion to each participating laboratory. The laboratories are aware of this program, test the material when it arrives, and send their reports to the sponsoring group. When all the results are received, the sponsoring group calculates the mean result reported by all participating laboratories and reports back to the participants their performance by means of a standard deviation index (SDI). This is an indication of the participating Laboratory's performance in terms of standard deviations from the mean result of all laboratories. An SDI greater than ±2 indicates that the Laboratory does not agree well with most of the others testing the same specimen.

As its name suggests, a blind proficiency test is identical to the open test, except that the participating laboratory is unaware of the test. The proficiency specimen arrives at the laboratory exactly like other subject specimens and is processed, tested, and results reported in a routine fashion. The surrogate subject then reports results back to the sponsoring group, which compares and scores results as before. Unfortunately, laboratories that do well in open proficiency testing sometimes perform poorly in blind testing (Hansen et al. 1985). Laboratory users should be aware of this difference in proficiency testing programs and attempt to identify laboratories willing to share their performance on blind PT programs before contracting for their services.

In order for. QA programs to work for the purpose for which they are designed, a continuous review process must be carried out. This keeps the laboratory director and supervisors constantly alert to the many variables that influence the results of chemical testing. In addition, it enables appropriate action to be carried out before errors are reported.

Excellent QA is costly, since personnel time, equipment, and supplies must be committed to it. It is essential, however, when test results may be used in decisions affecting employment, reputation, or even imprisonment of the subjects being tested.

SOURCES OF ERRORS

Scientists recognize that errors may occur during any type of scientific measurement. It is for this very reason that sound quality assurance programs must be implemented and carried out. In this way, random errors can be identified and corrected before reporting an analytical result.

Responsible critics have identified those aspects of urine drug testing that are vulnerable (McBay 1966; Hanson 1986). This discussion centers on human errors, errors in methodology, and alternate interpretations.

Human Errors

Errors of omission as well as commission occur in all human activities. Fatigue, poor health, and boredom arising from the tedium of routine tasks all contribute to high error rates. Providing good working conditions, effective rest periods, and rotation of workers through different tasks can help to alleviate these problems. Automation can minimize human error, provided automated steps arc monitored by an effective QC program, which is more easily accomplished in a laboratory with a high workload than in a small laboratory.

Inappropriate training or experience for the task being carried out can also lead to errors. It is for this reason that personnel qualifications, discussed earlier, must become part of the total QA program.

The most difficult errors to control are administrative ones. Labeling errors, spelling errors, transposition of numbers, all can lead to a correct test result being assigned to the wrong subject. In fact, most laboratories have learned by participating in external PT programs that these occur more frequently than errors in testing procedures. For this reason, the steps involved in specimen acquisition, transport, and processing (chain of custody) must be part of the complete QA program. Drug testing laboratories must practice good personnel policies like any other well-run enterprise.

Errors of Methodology

These types of errors have been discussed earlier relative to QC procedures. Instrument malfunction, outdated reageuts, measurement errors, and many other factors must be controlled by an effective QA program.

It is important to emphasize that testing procedures cannot be forced to yield results for which they are not designed. In particular, screening tests must be confirmed.

Chromatographic procedures may not resolve substances sufficiently for clear identification, while immunoassay procedures may use antihodies that cross-react with a variety of substances. Recently, it was reported that an immunoassay procedure, EMIT-d.a.u., erroneously gave a false-positive result for amphetamine in the presence of the antihypertensive agent lahetalol. Similarly, a commercial TLC screening procedure, TOXI-LAB A, confused the same antihypertensive agent with amphetamines or trimethoprim (Apple et al. 1985). Although the TONI-LAB manufacturer has indicated that resolution of these substances can be made (Martel et al. 1986), the incident illustrates the necessity for confirming all screening tests.

Other examples of false-positive results due to cross-reaction of immunoassays have been reported. The popular antihistamine diphenhydramine can react with the EMIT-d.a.u. antibody for methadone (Kelner 1984). Recently, Syva notified users of the EMIT kits for marijuana that the analgesic ibuprofen (available over the counter), as well as some other nonsteroidal anti-inflammatory agents, can interfere with the EMlT test for marijuana (at the 20 ng/ml cutoff level), although the company has reportedly taken steps to eliminate the problem by alteration of the enzyme reagent in the system. Other interferences have been reported by Allen and Stiles (1981).

These types of false-positive testing results are not due to deficiencies in methodology. Rather, they are the result of using screening tests without adequate confirmation. In each case cited, effective confirmation procedures will prevent the erroneous reporting of false-positive results.

RETENTION OF POSITIVE SPECIMENS

Since the possibility of error exists, although this is unlikely when appropriate confirmation is done, excess urine from all positive specimens should be retained until the case has been resolved. The remaining specimen in its properly labeled, original container should be stored by freezing (-20°C or lower). This will permit reanalysis by the original laboratory or a different laboratory in the event the positive results are challenged by the subject. The time between the original testing and ultimate resolution of the matter can be quite variable, depending on the circumstances. For this reason, clients should inform the laboratory as to when ultimate disposal of the specimen can occur.

DIFFERENCES IN INTERPRETATION

Finally, the testing procedure may produce results that accurately indicate the presence of drugs or their metabolites in a urine specimen, but explanations other than drug abuse may be invoked. These are not truly errors but rather alternate interpretations of the accurate test results. Frequently, these interpretations are invoked in the event that a charge is brought against a subject whose urine test has been reported as positive.

Many examples can be mentioned, most of which are related to marijuana. Accidental or inadvertent ingestion of marijuana as salad greens or components of food has been offered as an explanation. While experimental evidence is not available to support this claim for marijuana in uncooked foods, studies have demonstrated the possibility of this occurrence in baked goods (Cone and Johnson 1986). Passive inhalation of marijuana smoke is more frequently raised as a defense. The latter phenomenon has been investigated. Although passive inhalation can give rise to metabolites of THC being excreted and measured in urine, it would be extremely unlikely that high concentrations can occur by this means of dosage (Perez-Reyes et al. 1983).

Recently, cocaine has been identified in certain herbal teas (Siegel et al. 1986). It is possible, therefore, that a positive test for cocaine may be obtained in urine from a subject who ingests large amounts of this material. Note, however, that such teas are technically illegal and have recently been removed from the market by Federal authorities.

Other alternative explanations of positive drug findings are based on claims of errors arising from screening tests. These have already been discussed. Effective testing procedures in which positive screening results are confirmed by valid tests will refute these claims. The presence of a specific drug or metabolite will be documented, although how or why the substance was absorbed by the subject may not be answered.

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Interpretation of Urinalysis Results

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Urine drug testing has been used for several decades to monitor human drug use. This type of urinalysis procedure was first used for patients who had been using drugs on a regular basis, and positive results were an indication of continued drug abuse. The methodology most commonly used for performing the tests, thin-layer chromatography, was satisfactory for detecting a wide variety of drugs in common use at that time.

Over the years, both the population being tested and the laboratory methodology have changed dramatically. Today, the use of urine drug tests has been extended outside the substance abuse treatment facility and is currently used as part of preemployment physicals and for monitoring drug use in employees in a variety of industries and government agencies.

From an analytical standpoint, the development of immunoassay procedures has provided inexpensive and very sensitive procedures for analyzing for classes of drugs in urine. The analytically powerful technique of gas chromatography-mass spectrometry has become more commonly available and is currently the method of choice for the analysis of drugs in a sensitive and specific manner and for confirming the presence of drugs found in an initial screen. Other methodologies such as gas-liquid chromatography and high-performance liquid chromatography also play a role in the analysis of drugs in urine. The variety of methodology available permits inexpensive mass screening and also allows the specificity needed to provide accurate and sensitive confirmation of drugs found in the initial screening test. The wide variety of methodology now in use, as well as the variety of drug use patterns, makes the interpretation of urine drug assays more complex.

Although the vast majority of all drug tests will be negative, the user must understand that both negative and positive test results have meaning. In order to understand how to properly interpret the results of a drug assay, one must first determine what information is desired. In addition to finding a reputable laboratory with adequate professional staff to assist them, consumers must define their individual goals for starting the program. In particular, they must determine the consequences that will befall individuals who test positive. Since no uniform required standards exist for urine drug testing, the use of expert consultants to assist in the development of a program will be beneficial.

Before embarking on any urine drug testing program, the consumer must first determine the program goals.

Some questions that might be asked about the meaning of a drug screen follow. If an analysis is positive, does it mean that:

- the subject is using the drug chronically?
- the subject is using the drug intermittently?
- the subject is addicted to the drug?
- the subject is taking the drug under a physician's order?
- the subject was under the influence of the drug when the urine was collected?

If, on the other hand, the analysis was negative, does it mean that:

- the subject has never used the drug?
- the subject may use the drug intermittently but has not used the drug recently?
- the subject knew that the urine would be screened, and stopped taking the drug long enough for the urine specimen to be negative?
- the subject diluted the urine at the time of collection, or prior to collection, by drinking large amounts of fluids prior to sample collection and thereby rendered it negative?
- the subject adulterated the urine by adding water or another substance or switched it with urine from another individual during the confusion inevitable when large numbers of persons are involved in a process like urine collection?

All these possibilities must be considered as part of the process of deciding the relevance of urine drug test results.

The end user of the results of a urine drug analysis must also be familiar with proper specimen collection and handling procedures, have a general understanding of laboratory methodology used to perform the analysis, and have some understanding of drug kinetics. This information will permit correct interpretation of laboratory results and allow for reconciliation of these data with the subject history.

The purpose of this chapter is to provide the reader with information that will help in such interpretation.

DRUG FACTORS THAT DETERMINE WHETHER A TEST IS POSITIVE OR NEGATIVE

Dose

The higher the dose of drug taken, the more likely that the drug will be detected in urine. As an example, a dose of 30 mg codeine might be detected for 1 to 6 hours after use by a particular method; a 60 mg dose by the same method might be detected for 1-10 hours.

Frequency of Use

Drugs remain in the body for varying lengths of time. Drugs like cocaine are eliminated from the body relatively rapidly. Depending on the assay

procedure, a single dose of cocaine, for example, may only be detectable in urine for 1 day or less. Continued use on a daily basis may cause the drug to be detectable for 2 or 3 days after cessation of use. Smoking a single marijuana cigarette may result in a positive urine test for cannabinoids for 1 or 2 days with some methods and for 3 to 5 days with more sensitive methods. Continued use of marijuana on a daily basis however, may result in a positive urine for 3 or more weeks after drug use has stopped. As a general rule, most drugs tend to accumulate in the body if they are taken on a regular basis. The more frequently a drug is ingested, the more likely that it will be detected on a urine drug test.

Time From Drug Use to Urine Collection

Different drugs leave the body over various time intervals depending on the drug, the dose administered, and the frequency of drug use. For drugs like cocaine that leave the body very rapidly, it is necessary to collect the urine very close to the time of drug use to get a positive sample. With drugs like marijuana that are eliminated more slowly, the urine collection could occur up to several days after use and still be positive.

Sensitivity of the Urinalysis Test Used

Laboratory tests used to detect drugs in urine have different sensitivities for various drugs. Immunoassays, for example, generally can detect smaller quantities of drug in urine than can thin-layer chromatography, If the assay is not sensitive, drugs will not readily be detected in the urine specimen. If tbe assay is extremely sensitive, it may detect drugs in urine for days or weeks after their use. By understanding the sensitivity of the assay, the client will be able to relate the assay result to a "drug use window" and thereby determine approximately when the drug could have been used.

The sensitivity of the first urine drug test, often referred to as a preliminary screen, is important for determining whether a urine is positive or negative. The most commonly used laboratory procedures for the initial screen are thin-layer chromatography (TLC), radioimmunoassay (RIA), the EMIT test, and the newly introduced TDx Abused Drug Detection Assays (TDx). Although the RIA, EMIT, and TDx tests use methodologies that are significantly different from each other, they have in common several features. Each of the tests assays for a specific drug or for drugs in a single class (e.g., methadone, barbiturate class, amphetamine class, cannabinoids, etc.). The tests are very sensitive and will generally detect drug use for 1 or more days after a single use. The TLC system has the advantage that it can detect a wide variety of drugs, but it has less sensitivity than the immunoassay tests. Ideally, a combination of the TLC and immunoassay tests could be used for optimum sensitivity and versatility. If costs are a significant factor, then the number and variety of different tests will be limited.

As an example, initial screening could include a TLC assay and immunoassays for drugs that are not detected well (or at all) by the TLC screen, but are known to be commonly abused. A typical test battery might, include the combination of a TLC screen, a cannabinoid immunoassay test, and a cocaine immunoassay test.

In addition to TLC and the immunoassay procedures, other laboratory tests that can be utilized include gas-liquid chromatography, high-performance liquid chromatography, and gas chromatography-mass spectrometry. These tests are rarely used as initial screens. They have the advantage that they can detect a wide variety of drugs and are generally more sensitive than TLC. Their primary disadvantages include relatively high cost and long assay times. For these reasons, they are used primarily for specific drug analyses and for confirmation (particularly GC/MS) of presumed positives from initial screens.

If the results of the preliminary screen are negative, no further chemical testing of the urine specimen is usually performed. If the preliminary screen is positive for one or more drugs, however, a second laboratory test is then necessary to verify the presence of the detected drugs. This "confirmatory" test must use different technology, must be as sensitive as or preferably more sensitive than the preliminary screen, and must be specific for the drug or drugs detected in the initial screen. The confirmatory test is usually performed by gas-liquid chromatography, high-performance liquid chromatography, or ideally by gas chromatography-mass spectrometry.

RELATING DRUG FACTORS TO INTERPRETATION OF THE URINALYSIS TEST

Negative Test Result

Several interpretations are possible:

- The subject is not using a drug that can be detected by the test.
- The subject may be taking one of the drugs detected by the test but
  - -is not taking a large enough dose to be detected
  - -is not taking it frequently enough to be detected
  - -the urine is being collected too long after the drug was ingested
  - -the urine sample was diluted or otherwise tampered with or switched with another sample by the subject.
- The subject may be taking the drug but the assay used was not sufficiently sensitive to detect the drug in urine.

Positive Test Result

If the test was properly performed and the results from the laboratory are valid, the result means that the drug indicated was present in the urine. A positive result does not mean that the individual tested was under the influence of the drug at the time the specimen was collected. An expert would be able to provide some general information regarding the timeframe of the drug use. Even experts will vary in their opinions, however. The significance of a single positive urine drug test can be increased by repeat testing at regular intervals. Multiple positives over a period of time reinforce that an individual may be a regular user of the detected substance. Although multiple negatives do not completely negate drug use, they do provide evidence that the individual may not be using on a regular basis.

Prolonged Monitoring

In some cases, such as treatment situations or probationary periods for an employee, it may be advisable to collect sequential specimens over time to assess continued drug abstinence.

Collection of multiple urine specimens over a prolonged period of time requires that special attention be given to subjects who have ingested drugs such as marijuana and certain benzodiazepine antianxiety agents that are excreted in urine for long periods of time. If the subject had used one of these drugs chronically prior to the start of urine drug testing, it is possible that the test could be positive for several weeks or months after he or she stopped using the drug.

Since the results of urine drug tests are normally reported as either positive or negative, it is not possible to determine if a positive result is due to continued use of the drug or continued excretion of the drug from previous use. In order to differentiate between recent drug use and continuing urine drug excretion from previous use, it is necessary to request that the laboratory perform a semiquantitative analysis for the drug in the urine. If the subject has stopped using the drug, the concentration of drug in urine would be expected to decrease each time the urine is assayed. If the concentration of drug increases, continued use of the drug should be considered.

Monitoring this decreasing drug level is complicated by variations in urine water content. Greater than normal intake of water can dilute the amount of drug in the urine, and, conversely, a limited intake particularly in conjunction with dehydrating exercise can lead to an abnormally concentrated urine specimen.

Figure 1 illustrates a series of samples taken every 2 days from one subject. The solid line is urine concentration for cannabinoids. The concentrations are seen to decrease over the 3-week period with the exception of an increase on day 6, which might imply a smoking incident.

When using the semiquantitative analysis procedure, it is imperative that the concentration of drug found in the urine be adjusted for the changes in the urine water content that occur throughout the day. The adjustment can be made by performing a urine creatinine analysis and dividing the drug concentration (ng/ml of urine) by the creatinine concentration (mg creatinine/ml of urine). This allows the drug concentration to be expressed as ng drug/mg creatinine. After drug use has stopped, measurement of ng/mg creatinine will show a continuous decrease on sequential sample analysis, while sequential concentration measures (ng/ml urine) may show significant fluctuation. Because creatinine is excreted at a relatively constant rate, the use of the creatinine analysis compensates for (or corrects) the hour-to-hour concentration or dilution of urine that normally occurs.

The need for this adjustment can be seen in the example in figure 1. The dashed line shows the excretion levels in terms of ng/mg creatinine. As can be seen, the apparent increase in excreted cannabinoids at day 6 is, in fact, a more concentrated urine specimen and not an indication of further marijuana use. On the other hand, the creatinine-corrected levels show a clear increase in excreted cannabinoid at day 10, indicating that the subject probably used marijuana again. This was not apparent in the ng/ml analysis. Apparently at

day 10, the urine was relatively dilute (perhaps in an attempt by the subject to mask the drug use), but the creatinine analysis provided the compensation for an appropriate interpret at ion.

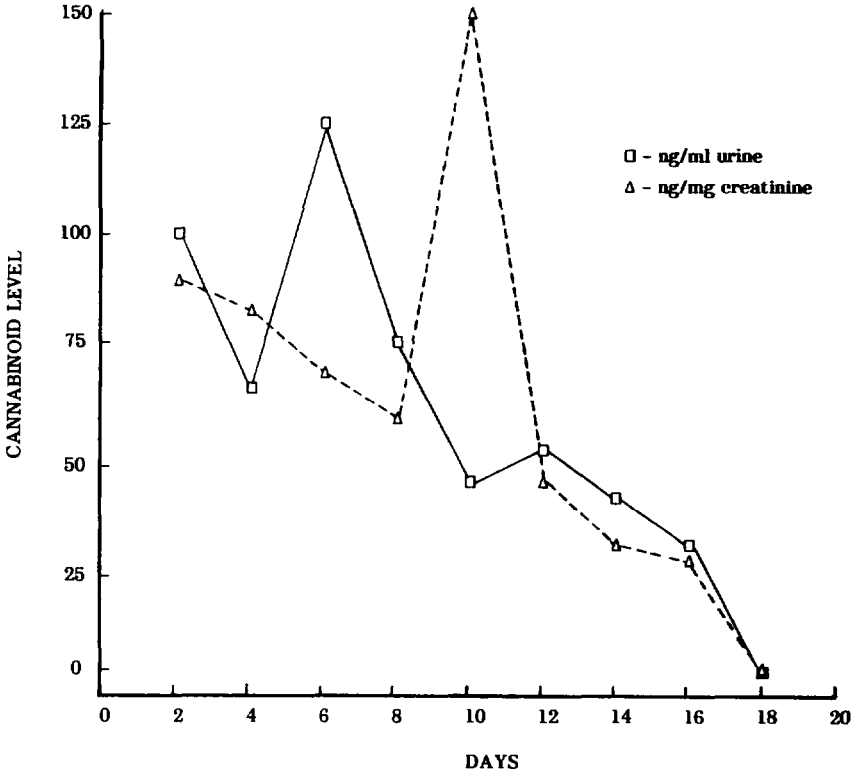

Figure 1. Urinary cannabinoid levels of specimens taken on alternate days after last marijuana use. □ – concentration of THC metabolite in ng/ml urine. Δ – THC metabolite concentration divided by the creatinine concentration expressed in ng metabolite/mg creatinine.

In this hypothetical example, several benefits were obtained by performing semiquantitative analyses and adjusting by the use of creatinine levels. The semiquantitative results allowed more careful monitoring than the positive or negative (concentration analysis) results. In the example shown, adjusting the cannabinoid concentration by means of the creatinine analysis protected the subject on day 6 by substantiating his story about sweating (which could have led to dehydration and subsequent concentration of the urine). On day 10, it allowed the detection of smoking and the subject's attempted coverup by sample dilution.

When used attentively, semiquantitative urine drug analysis and adjustment with creatinine can provide a valuable adjunct to the interpretation of multiple urine drug screens taken from healthy individuals who have abused drugs that are excreted slowly from the body.

METHODS OF URINALYSIS

It is important to remember that no single laboratory test is capable of detecting all drugs in urine. The commonly used term "drug screen" is somewhat inaccurate in that it implies that all drugs are detected. Quite frequently, laboratories will provide lists of drugs that can be detected by their particular drug screen. Even with such a list, the consumer must still be cautious unless the list specifically details which drugs are detected in urine. For purposes of this discussion, the term "comprehensive drug screen" will be used to describe tests that can detect a variety of drugs in urine. Other laboratory tests that can detect individual or classes of drugs will be referred to as special or specific tests.

Comprehensive Drug Screen

The comprehensive drug screen can be performed by a variety of laboratory techniques. While gas-liquid chromatography, gas chromatography-mass spectrometry, and high-performance liquid chromatography are sometimes used for this purpose, thin-layer chromatography remains the most commonly utilized technique for the detection of a large number of drugs at low cost. The other chromatographic techniques are used to a much more limited extent because of higher cost per assay. They are generally employed for specific drug assays and for confirmation purposes.

It must be understood that considerable variation does exist between laboratories in the performance of this test, a factor that will influence both the types of drugs detectable and the time interval during which they can be detected. Detection times are also affected by the dose of the drug, the frequency of use, the time from drug use to urine collection, and the specific laboratory procedure used for performing the test.

The following drugs can be detected by comprehensive drug screens based on TLC procedures for periods of 24 hours or longer after use:

- Amphetamines such as amphetamine, methamphetamine, pseudoephedrine, phenylpropanolamine, and ephedrine
- Benzodiazepines such as chlordiazepoxide (Librium), diazepam (Valium), and flurazepam (Dalmane)
- Barbiturates such as phenobarbital, secobarbital, and amobarbital
- Methadone
- Propoxyphene (Darvon)
- Tricyclic antidepressants such as imipramine, desipramine, etc.
- Nicotine

Drugs that can be detected for shorter periods of time after use, usually 3 to 12 hours, include:

- Opiates such as morphine, codeine, etc.
- Pentazocine (Talwin)
- Cocaine

Drugs that cannot normally be detected in urine by a TLC comprehensive drug screen are:

- Cannabinoids (marijuana)
- Phencyclidine (PCP)
- LSD and other hallucinogens

Specific Tests

Specific tests include EMIT, RIA, TDx, GC, HPLC, and GC/MS. The specific urine tests that are available are usually more sensitive than thin-layer chromatography, but they can generally be used to test for only one drug or class of drugs in a single assay experiment. Generally, a subject will be positive with one of the specific urine tests for 1 to 3 days after drug use. These tests are most commonly performed using immunoassay procedures. Because they are restricted to individual drugs or classes of drugs, desired tests must be specifically ordered. The option includes tests for:

- Alcohol (ethanol)
- Amphetamine
- Barbiturate
- Benzodiazepines [detects various benzodiazepines, including chlordiazepoxide (Librium), diazepam (Valium), and flurazepam (Dalmane)]
- Cocaine
- Methaqualone (Quaalude)
- Opiates (detects several of the opiate drugs, including morphine, codeine, and hydrocodone [Dilaudid] for a day or more after use)
- Methadone
- Phencyclidine (PCP)
- Propoxyphene (Darvon)
- Cannabinoids (THC metabolite--detects marijuana use)

DRUG TESTING STRATEGY

It can be seen that a urinalysis can include a single test or a battery of different tests. In order to develop a drug testing program that serves the needs of the client at a reasonable cost, individualized test batteries must be carefully selected. In some cases, a general or comprehensive screen with followup confirmation testing may be appropriate where the client or employer is willing to sacrifice some sensitivity in order to screen for a wide variety of drugs. In other cases, an analysis program aimed at three or four drugs that are known to be problematic in a given work situation may be indicated. Increased sensitivity for a smaller number of drugs increases the likelihood of detecting these substances and decreases the chance of false negatives.

Implications of Drug Levels in Body Fluids: Basic Concepts

C. Nora Chiang, Ph.D., and Richard L. Hawks, Ph.D.

Urine drug analysis aimed at the detection of drug users generally is focused on the detection of illicit drugs such as marijuana, cocaine, amphetamines, etc., or their metabolites To effectively use the results that can be provided by current technology for the analysis of biological fluids, a basic understanding of drug pharmacokinetics and pharmacodynamics is important (Rowland and Tozer 1980; Holford and Sheiner 1981; Gibaldi and Perrier 1982; Goodman and Gilman 1985). Such an understanding provides the basis for attempts to estimate the time and extent of drug use and possibly the extent to which the drug level is predictive of impairment or pharmacologic effect. Such knowledge is equally important in understanding the limits of interpretation of drug levels in specific instances.

Pharmacodynamics describes the relationship between the concentration and the effect of a drug. Drug effects result from the presence of appropriate drug concentrations at the site of drug action To reach the site of drug action, except for a few drugs that act topically, a drug must enter the blood circulation from the site of administration. Through blood circulation, the drug distributes to the site of drug action and simultaneously to other tissues where it is stored, metabolized, and excreted. The fate of a drug in the body is schematically represented in figure 1. The concentration of most drugs at the site of action is therefore a function of the drug concentration in the blood. The concentration or amount of a drug in other body fluids such as saliva and urine can, in some cases, be related to drug concentration in the blood. Pharmacokinetics is a science that quantitatively describes these processes--absorption, distribution, metabolism, and excretion--that a drug undergoes in the body.

This chapter describes basic principles of pharmacokinetics and pharmacodynamics and the application of this information to the understanding of the significance of drug concentrations in terms of prediction of time of drug exposure or degree of drug effects. Marijuana and cocaine will be used as specific examples to illustrate these principles.

PHARMACODYNAMICS

The effects of most drugs result from interactions with their receptors at the site of drug action. The time course of most responses, determined by the effective concentrations of the drug at these receptor sites, depends on the processes that a drug undergoes in the body. As plasma concentrations generally reflect drug concentrations at the site of drug action, the intensity of the effects for most drugs can be related to plasma concentrations, although this relationship is usually not a simple linear one.

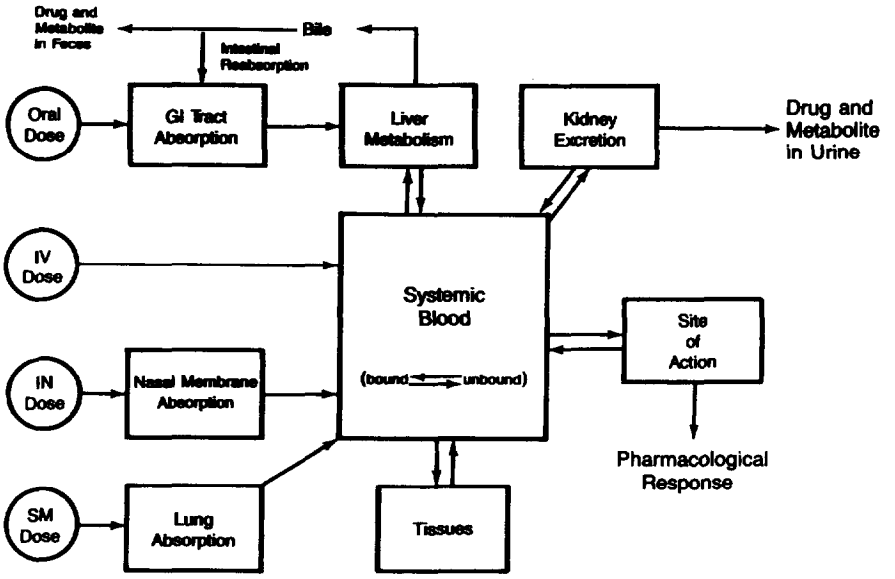

Figure 1. Schematic representation of the fate of a drug in the body.

A poor correlation is often found between effects and plasma drug concentrations. Factors that may contribute to the poor correlation are the presence of active metabolites, time delays in the drug response, or the development of tolerance. The relationship of specific drug concentrations to effects for a general population is further complicated by individual differences in the pharmacological response and the pharmacokinetics of the drug.

Most abused drugs act on the central nervous system and produce effects on mood, perception, behavior, and performance (Goodman and Gilman 1985). The relationship of a perceived subjective "high" and performance decrements with plasma concentrations has been a subject of many studies.

Psychological "High" Effects and Drug Concentrations

Pleasurable effects have been associated with the self-administration of psychoactive drugs such as cocaine and marijuana. The time course of the effects produced by cocaine and plasma levels after administration of cocaine by the intravenous (20 mg), intranasal (95 mg), and smoking (50 mg) routes are shown in figure 2 (Cook et al. 1985). In spite of the twofold difference in the dose for intravenous and smoking routes of administration, the plasma profiles of cocaine are very similar, as are the profiles for the self-reported "high" effects. Following the intranasal route of administration, the plasma cocaine levels peaked more slowly and were prolonged longer as compared with those following the intravenous and smoking routes. The high effects reported by the subjects were also perceived at a later time point for the intranasal dose. This result indicates that the high effect is

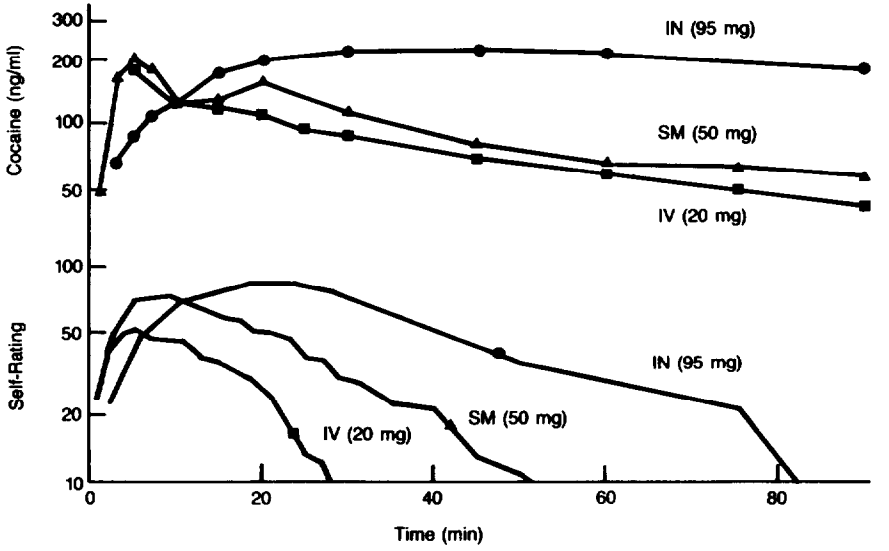

Figure 2. Comparison of cocaine plasma concentrations (upper panel) and psychological self-reported "high" (lower panel) after administration of cocaine by intravenous, intranasal, and smoking routes (From Cook et al. 1985. Copyright 1985, PSG Publishing Company, Inc.).

better related to plasma cocaine concentrations than to the dose administered. The decline of the high effect is more rapid than the decline of plasma cocaine levels. Tolerance to the subjective high effects has been suggested (Fischman et al. 1985).

In the case of the psychoactive component in marijuana, delta-9-tetrahydrocannabinol (THC), the pattern of the high effect showed a slower rise and a slower decline than the plasma level of THC (figure 3) (Hollister et al., 1981; Chiang and Barnett 1984; Ohlsson et al. 1985). This delay of the high effect suggests that the site of action of THC is not easily accessible to the blood. When this was taken into consideration in a pharmacokinetic and pharmacodynamic compartmental model analysis, the effects were able to be well correlated with plasma THC levels (Chiang and Barnett 1984). However, the estimation of the degree of intoxication from a single value of THC plasma level is very difficult (Hollister et al. 1981), due not only to this time delay but also to large individual variations in both effects and plasma levels.

Performance Decrement and Plasma THC Concentrations

Marijuana use has been associated with decrements in behavior and cognitive performance. The incidence of detectable levels of THC in fatally injured drivers ranges from 3.7 percent to 37 percent (Mason and McBay 1984; Williams et al. 1985). While a direct causal effect of marijuana is difficult to establish from such studies because of the generally high incidence of alcohol

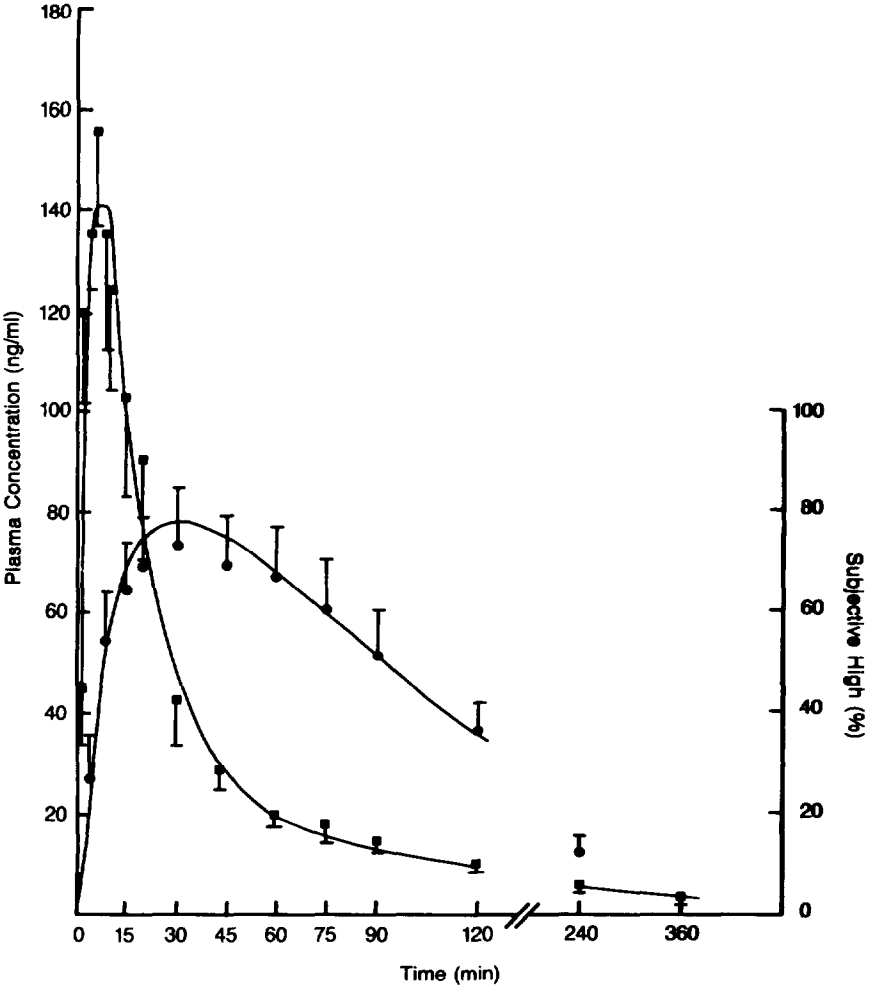

Figure 3. Plasma THC concentration-time (■) and subjective high-time (•) after smoking one 2.5 percent THC cigarette (mean ± SE; n=6) Solid curves are computer fits to the data (From Chiang and Barnett 1984. Copyright 1984, The C.V. Mosby Co.)

in the subjects as well as lack of control groups, the indication is clear that a performance-impairing drug (THC) is present and is therefore likely to contribute to the factors leading to the accident.

In laboratory investigations, it has been reported that THC may affect complex performance skills up to 24 hours after drug ingestion. One study reports a "hangover" effect on the morning following a dose of marijuana (9

hours after smoking a marijuana cigarette of 3 percent THC) (Chait et al. 1985). The mean performance of pilots in a flight simulator showed a trend toward impairment at 24 hours after smoking a cigarette of 2 percent THC (Yesavage et al. 1985). These results have implications for THC effects on the performance of complex tasks the day after smoking when blood concentrations are very low.

A recent study demonstrated that a mathematical correlation could be established between certain laboratory tasks and THC plasma concentrations (Barnett et al. 1985). This correlation was shown for the "critical tracking breakpoint" task for up to 7 hours after smoking and for plasma THC concentrations as low as 2 ng/ml. This and similar tasks are widely used to evaluate factors related to driving; however, the precise predictive validity is unknown. Although impairment on a laboratory task can be related to plasma THC levels, the extent to which it predicts driving impairment from smoking marijuana is unclear.

Presumptive impairment levels for THC are difficult to establish, although the impairment for alcohol has been reasonably correlated with blood alcohol levels. Data currently available indicate that wide ranges of drug concentrations for different individuals may be present at equal levels of impairment (Reeve et al. 1983). Conversely, evidence of impairment is often lacking in some subjects at drug concentrations that are associated with impairment in others. A general consensus of forensic toxicologists stated that the blood concentrations associated with impairment after smoking marijuana and after use of many other drugs has not been sufficiently established to provide a basis for legal testimony in cases concerning driving under the influence (Consensus Report 1985).

PHARMACOKINETICS

Pharmacokinetics, which characterizes the relationship of drug concentrations with time, is important for the understanding of drug actions. It is a quantitative description of the complex processes that a drug undergoes in the body, as shown in figure 1. From the site of administration, a drug is absorbed into the systemic circulation. Through circulation, the drug is distributed into various tissues. At the site of action, it exerts its pharmacological effects. It may also accumulate in tissues. It is eliminated from the body through metabolism or excretion by the eliminating organs. (The liver is the primary organ for metabolism and the kidney the most important organ for excretion for most psychoactive drugs.) The concentration of a drug in the body (pharmacokinetic profile) is a function of these processes--absorption, distribution, metabolism, and excretion (Rowland and Tozer 1980; Gibaldi and Perrier 1982; Goodman and Gilman 1985).

Absorption

Absorption describes the transfer of a drug from the site of administration into the systemic circulation (figure 1). Drugs can be administered by various routes, which are classified as intravascular and extravascular.

Intravascular administration refers to a direct and instant input of a drug into the systemic circulation and is usually accomplished by intravenous administration of a bolus dose or by an infusion. The intravenous route of administration is commonly used for heroin, methamphetamine, and cocaine. For a given dose, the shorter the time of infusion, the earlier and higher is the maximum concentration in the blood. The onset of the pharmacological response is also more rapid, and the intensity is greater. This increases the risk of toxic effects of the drug.

By extravascular routes of administration such as smoking, intranasal administration, or oral ingestion, the drug gets into the systemic circulation through absorption processes. The rate and extent of the absorption of the drug from different routes may be quite different, depending on the physicochemical properties of the drug as well as the physiological and anatomical factors involved in absorption.

From the smoking route, the drug is inhaled and absorbed from the lung or the respiratory tract into the circulation. The amount absorbed may be quite variable, as part of the dose is lost from pyrolysis and from side-stream smoke. The amount absorbed is affected by the burning characteristics of the cigarette, the depth of inhalation, and the puff duration. However, the rate of absorption from this route is in general very rapid. Peak plasma concentrations are rapidly reached, and the plasma profile is very similar to that of an intravenous dose. This route of administration is generally used for marijuana, phencyclidine (PCP), cocaine, and occasionally for heroin and methamphetamine.

The intranasal route of administration, in which the drug is absorbed from the nasal mucous membrane, is commonly used for cocaine and occasionally for heroin. A plasma concentration time curve for cocaine is shown in figure 2. As shown here, the intranasal route also provides a relatively quick and sustained level of drug in the plasma.

Oral administration, the most commonly used route for therapeutic drugs, is a relatively less popular route for abused drugs except for the hallucinogens, such as lysergic acid diethylamide (LSD), 3,4-methylenedioxymethamphetamine (MDMA or "Ecstasy"), and mescaline. The drug so taken is absorbed from the gastrointestinal (GI) tract. The absorption rate depends on the physicochemical properties of the drug, and it may not be completely absorbed. The drug may be degraded by gastric juice or the digestive enzymes, or it may also be metabolized by intestinal flora in the GI tract or the enzymes in the liver, which result in less drug entering the systemic circulation. Absorption may be affected by the presence of food or other drugs.

Figure 4 illustrates the plasma profiles of THC after administration by intravenous (IV), oral, and smoking (marijuana) routes. Plasma THC profiles for IV and smoking are similar, with peak levels reached rapidly and declining rapidly. After oral administration, plasma levels for THC increase slowly and the peak level is lower. This indicates that smoking provides an efficient method of delivering drug into the systemic circulation. However, the amount absorbed from smoking is quite varied, ranging from 2 percent to 56 percent of the amount of THC in the cigarette, depending on the efficiency of the smoker (Lindgren et al. 1981). Experienced smokers may inhale more efficiently than naive ones. The fraction of dose absorbed after the oral

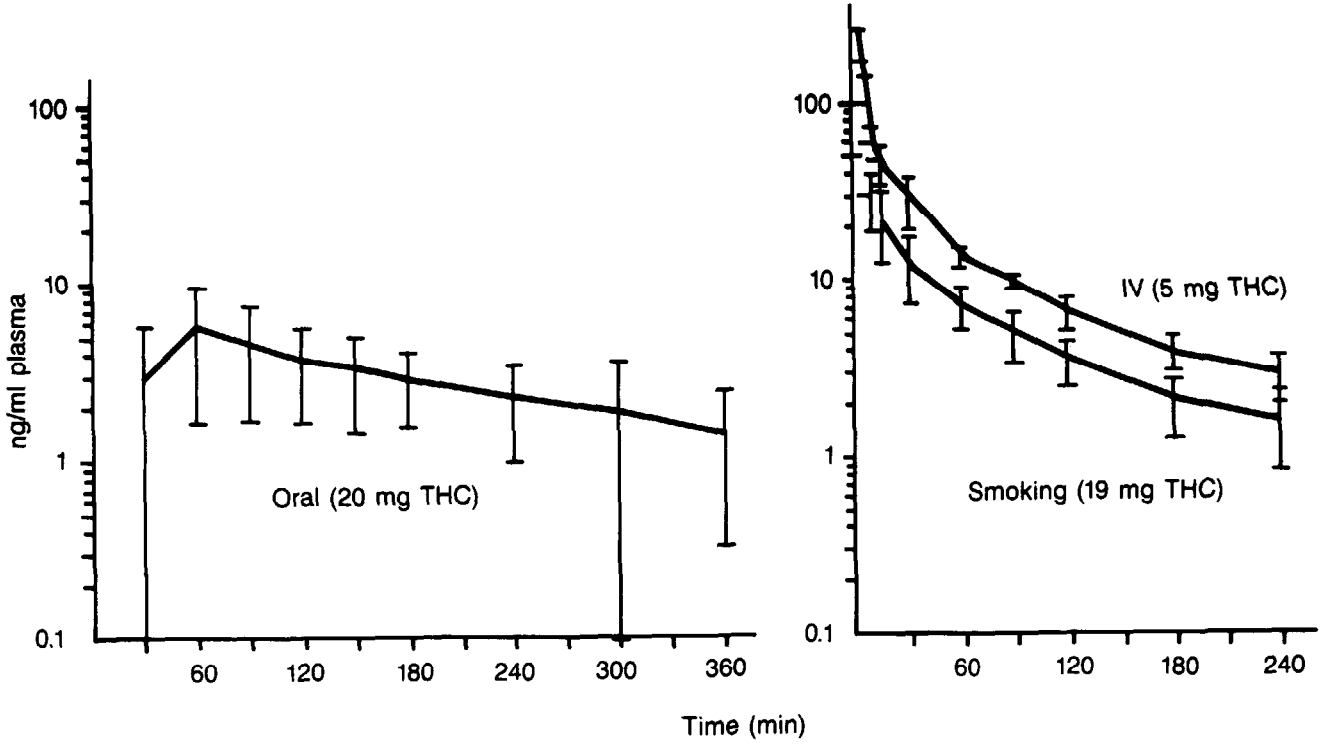

Figure 4. Plasma concentrations (means and standard deviations) of THC after intravenous, smoking, and oral routes of administration (From Ohlsson et al. 1980. Copyright 1980, The C.V. Mosby Co.).

route ranged from 6 percent to 20 percent (Ohlsson et al. 1980; Wall et al. 1983). Because THC is extensively metabolized by the liver, part of the drug may be degraded to metabolites before reaching the systemic circulation.

Distribution

Once the drug is absorbed into the systemic circulation, it is distributed into different tissues, into the site of action to elicit pharmacological effects, into organs where metabolism or excretion occurs, and into tissue reservoirs in which it may accumulate and serve as a storage depot of the drug. The rate and extent of distribution in tissue are determined by the blood flow to the tissue, the ability of the drug to pass through the tissue membrane, and the relative binding affinity of the drug for the tissue and plasma proteins. Storage of drugs in the tissue by tissue binding or dissolution in the adipose tissue may prolong the time in which drugs are detected in the plasma. Drugs such as THC are very lipophilic and distribute into tissues, including the adipose (fatty) tissue, leaving a small amount in the blood. The slow release of THC from the sequestered tissue including adipose tissue into the plasma is suggested for the reason for the slow elimination of the drug from the body and therefore the long detection times of previous marijuana use in urinalysis.

Metabolism

Drugs are eliminated from the body through metabolism and excretion. Liver is the primary metabolizing organ, although lung, kidney, and other tissues also have metabolic activities. The drug is metabolized by oxidation, reduction, hydrolysis, and conjugation reactions to more polar forms (metabolites) to facilitate the excretion process. Metabolism also provides a mechanism to terminate drug effects, as most metabolites are devoid of pharmacological activities. Some metabolites, however, rnay have pharmacological activities that are similar to or different from those of the parent drug.

Most psychoactive drugs are extensively metabolized. Over twenty metabolites of THC have been identified in urine and feces, including various carboxylated (acidic) and hydroxytated metabolites as well as glucuronic acid conjugates (Wall et al. 1981, Widman et al. 1985). Still, a significant portion of the dose in both urine and feces has not been identified. Metabolites in the urine are primarily the carboxylated products. The major metabotite is 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid (9-carboxy-THC), a non-psychoactive metabolite. The metabolites, 11-hydroxy-delta-9-tetrahydrocannabinol (11-OH-THC) and 8-beta-hydroxy-delta-9-tetrahydro-cannabinol are reportedly psychoactive (Lemberger et al. 1972; Perez-Reyes et al. 1972). However, they are found only in very low (frequently undetectable) concentrations in plasma after smoking or intravenous administration and are unlikely to make a significant contribution to the effects of THC. Their concentrations in urine as well as that of THC itself are frequently undetectable.

Cocaine undergoes primarily metabolic hydrolysis by the esterases present in liver and in plasma (Cook et al. 1985). The major metabolites identified in urine are benzoylecgonine, ecgonine methyl ester, and ecgonine. These metabolites are not psychoactive.

Excretion

Drugs are eliminated from the body as unchanged drug or as metabolites through urine, bile, sweat, saliva, and expired air. Renal excretion is a common and important route for drug elimination, making kidney the most important organ for drug excretion. For many drugs, biliary excretion also plays an important role. Through bile, drugs enter into the gastrointestinal tract, where they may be either excreted in feces or reabsorbed and excreted through urine or feces. Other routes such as saliva or sweat do not in general contribute significantly to the excretion of most drugs. They may be of forensic importance for the detection of drugs, however.

For THC, about 80 percent to 90 percent of the dose is excreted during the first 5 days following a dose. Approximately 65 percent of the dose is excreted in the feces and 20 percent excreted in urine (Hunt and Jones 1980; Wall et al. 1983). Cocaine is excreted almost exclusively in the urine. Only 4 percent to 6 percent is excreted in the feces (Cook et al. 1985). Both THC and cocaine, as well as most other drugs, are extensively metabolized. The urinary as well as fecally excreted compounds are primarily metabolites with only a minor fraction of unchanged drugs. Phencyclidine, on the other hand, is excreted unchanged to the extent of 10 percent (Cook et al. 1982).

Salivary excretion-- The excretion of a drug into saliva depends on the ability of the drug to pass through the epithelial cells of the salivary glands into the saliva. The concentration of a drug in saliva could be higher, such as amphetamine (Wan et al. 1978), or lower, such as methaqualone (Sharp et al. 1983), than that in plasma. THC or its metabolites are not detectable in saliva following an intravenous dose (Perez-Reyes, personal communications). THC is detectable after smoking marijuana, however, due to the absorption of THC by the oral mucosa during smoking. This illustrates the need for caution in the interpretation of saliva concentrations of drugs shortly after an oral or inhalation dose. Nevertheless, the THC in saliva after smoking is reported to be indirectly correlated with recent use of marijuana (Gross et al. 1985).

Saliva is perhaps the most easily obtained sample, and it has been established that some drugs can be easily detected in saliva (Danhof and Breimer 1978). It has not come to be generally used, however, because salivary concentrations for many drugs are so low that they cannot be detected. In addition, the methods of analysis for saliva samples are frequently more difficult than for urine samples. Furthermore, the time period during which drugs can be detected after use is usually shorter than that in the urine.

Renal excretion-- Most drugs are extensively metabolized in the body and excreted in the urine as metabolites. The processes involved with the excretion of a drug via the kidney are filtration, secretion, and reabsorption.

In the kidney, drugs that are in the “plasma water” (not bound to plasma proteins) pass through the glomerulus, which functions to filter the water as welt as small molecules and leave the protein material of the blood behind (along with any drug bound to it). This water filtrate then passes through the tubules of the kidney, where water is reabsorbed and thereby the filtrate become more concentrated. This “concentrated” filtrate leaves the kidney to the bladder as urine. In the kidney, acidic or basic drugs (or metabolites) can be added to the filtrate (urine) by the process of secretion through the

tubular membrane. Drugs or metabolites in the filtrate (urine) may diffuse back into the blood by reabsorption through the tubular membrane.

The filtration process occurs with all drugs and metabolites, but secretion and reabsorption do not occur with all drugs or metabolites. Since only unbound drugs and metabolites are filtered in the kidney, the extent to which a drug is protein-bound influences directly the rate at which it will be excreted in the renal system. The reabsorption process is affected by urinary pH as well as urinary flow, as are the excretion rates of some drugs and metabolites. For most drugs that are not actively secreted or whose concentration is far below the saturation concentration for secretion, urinary excretion rates are directly proportional to drug plasma concentrations. Plots of the urinary excretion rate and the plasma concentration of cocaine and benzoylecgonine are shown in figure 5 (Ambre 1985). The excretion rates of cocaine and benzoylecgonine are parallel to the respective plasma concentrations.

As the urinary flow rate and pH may change from time to time, urinary excretion rates could also be affected by these changes and may fluctuate. In some cases, a plot of excretion rates may not parallel that of plasma concentrations.

Urine drug analysis--Urinalysis quantitates the concentration of a drug in the urine. A change in the urinary flow rate may change the concentration of a drug in the urine, even if the renal excretion rate remains the same. ln general, drug in the urine is more concentrated than that in the plasma. For example, if a drug is neither secreted nor reabsorbed by the kidney, the concentration of the drug in the urine will be about 100 times that of the unbound drug in plasma. This is due to the fact that about 99 percent of the plasma water filtered into the tubular lumen is reabsorbed, and only 1-2 ml/min of water leaves the kidney as urine. Ingesting a large volume of liquid or using diuretics may increase the urine volume, and drug in that urine is therefore diluted. Creatinine is an endogenous substance that is neither secreted nor reabsorbed and can therefore be used as a "marker" to correct the fluctuation of urine flow. In some laboratories engaged in urinalysis, drug urine levels are reported as nanograms or micrograms of drug per milligram of creatinine (see the previous chapter).

PHARMACOKINETIC PARAMETERS

Pharmacokinetics is a mathematical description of the time course of a drug in the body. The important parameters are bioavailability, clearance, volume of distribution, and half-life. Bioavailability refers to the fraction of a dose that enters into the systemic (blood) circulation. Clearance indicates the ability of an organ to clear drug from the systemic circulation. The volume of distribution indicates the amount of drug in the body in relation to the drug concentration in the plasma or blood after equilibrium has been achieved. Half-life is the time required to reduce the plasma concentration by 50 percent.

Generally, the half-life is determined from plasma concentration measurements during the terminal or elimination phase (after distribution processes have equilibrated). As the clearance of a drug by an eliminating organ in

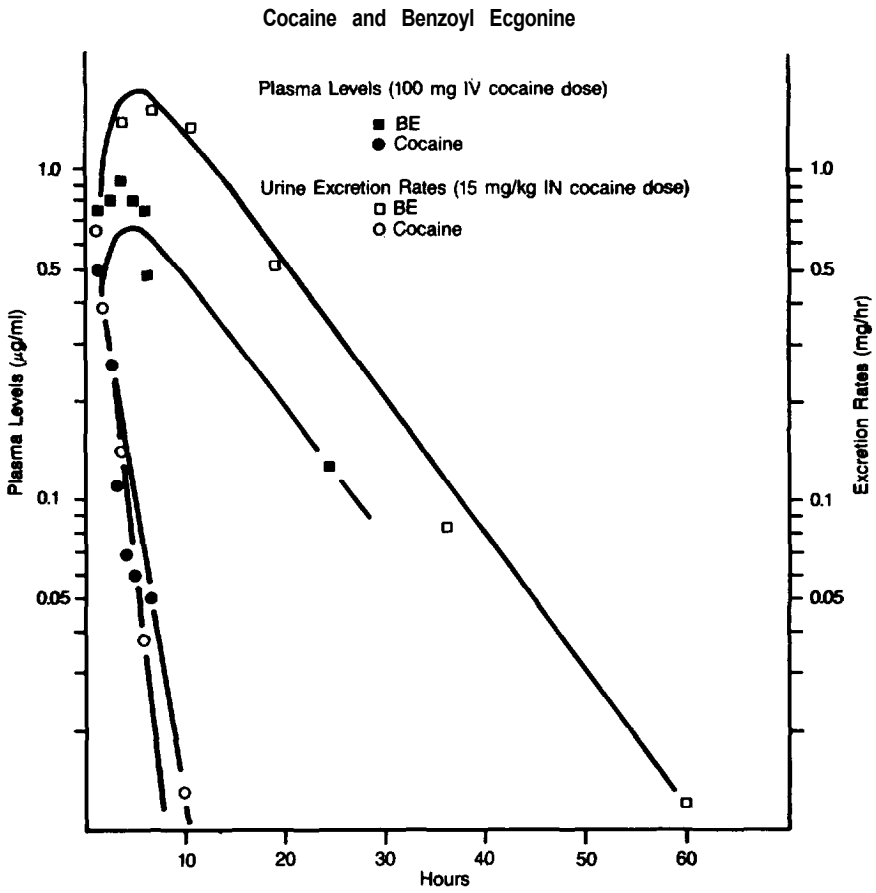

Figure 5. A similar plot of plasma levels and urinary excretion rates of cocaine and benzoylecgonine (From Ambre 1985. Copyright 1985, Preston Publications, Inc.).

general is constant, the rate of elimination of a drug is a function of the concentration of a drug in plasma. Plasma drug concentrations decline following a first-order rate constant--a constant fraction of the drug is eliminated per unit time. A half-life is the time unit for removing 50 percent of the drug from the body by either metabolism or excretion. A five half-life period is required to eliminate approximately 97 percent of the drug in the body.

Examples of pharmacokinetic analysis for cocaine (one-compartment model) and THC (multiple-compartment model) follow.

One-Compartment Model

Cocaine exemplifies a one-compartment pharmacokinetic model (figure 6). This model considers the body as a single homogeneous compartment and assumes that the drug which is introduced into the plasma is instantaneously in equilibrium with other tissues. Although the drug concentration in other tissues may not necessarily be the same as that in the plasma, any changes in the tissue are reflected by changes in plasma--the central compartment. The elimination of the drug in the body follows first-order kinetics (the rate constant k), and the elimination rate of the drug is proportional to plasma drug concentration.

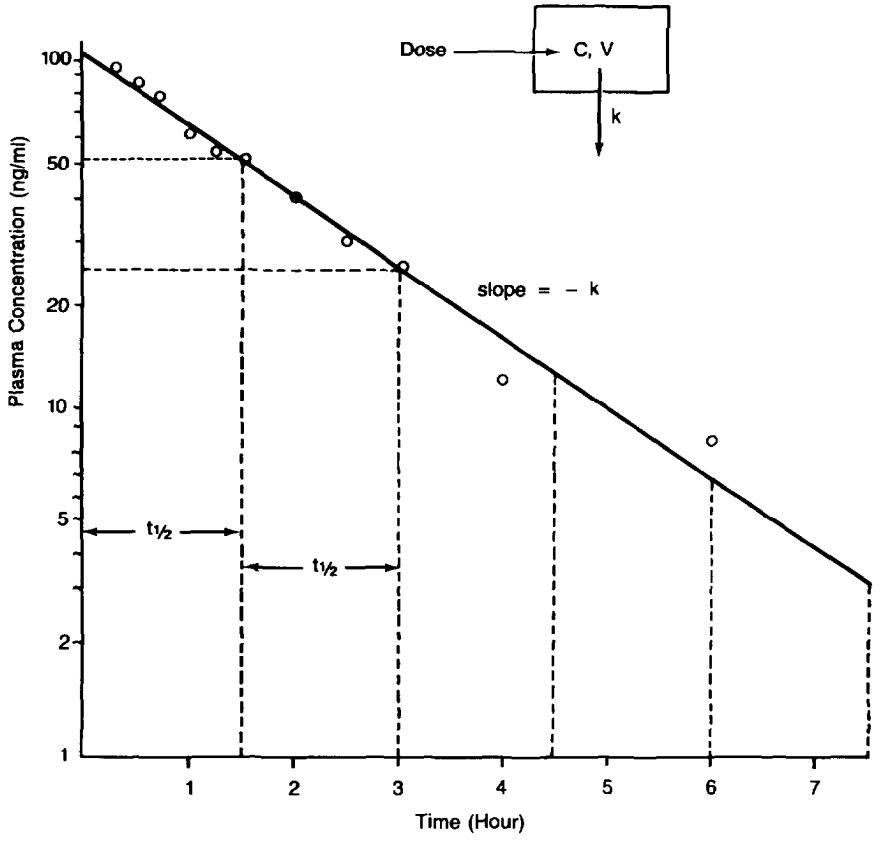

Figure 6. Plasma levels of cocaine (o) following an intravenous dose of 20 mg cocaine. Solid curve is a computer fit of the data to a one-compartment model (data from Cook et al. 1985). The inset is a schematic representation of a one-compartment model.

The plasma concentration (C) at a certain time (t) after an intravenous bolus dose is described by the following equations.

$$C = C_o \exp(-kt) \quad \text{(Eq. 1)}$$
$$V = \text{Dose}/C_o \quad \text{(Eq. 2)}$$
$$t_{1/2} = 0.693/k \quad \text{(Eq. 3)}$$

Where C_o is the plasma concentration extrapolated to time zero, k is the elimination rate constant, V is volume of distribution, and $t_{1/2}$ is half-life.

A plasma concentration-time curve for an intravenous dose of 20 mg cocaine in a subject is illustrated in figure 6 (data from Cook et al. 1985). The solid line is the least square fitted curve of the data using equation 1. C_o is estimated to be 102 ng/ml, and the elimination rate constant (k) is 0.46/hour. The volume of distribution (V) estimated by Eq (2) is 196 liters, which indicates that the majority of cocaine in the body is distributed in the tissue. The half-life from Eq (3) is 1.5 hour.

The half-life is a very important parameter for estimating the time required to eliminate the drug in the body. It takes one half-life for plasma levels to fall to half of their original level. In the case of cocaine (fig. 6), it takes 1.5 hour for cocaine plasma levels to fall from 102 ng/ml to 51 ng/ml. This is the same time that it takes for concentrations to fall from 51 ng/ml to 25.5 ng/ml. By five half-lives (7.5 hours) the plasma concentration of cocaine decreased from 102 ng/ml to 3.1 ng/ml, which is 3 percent of the original drug in the body. Almost all the drug (97 percent) will be eliminated by five half-lives. For reaching a certain drug level, an additional half-life will be required if the dose is doubled. For example, if cocaine is given at a dose of 40 mg, it will take two half-lives to reach the level of 51 ng/ml and six half-lives to reach the level of 3.1 ng/ml.

Mu1ticompartment Model

The disposition of most drugs, including THC, is described by a multicompartment model. Here, the body consists of a central compartment interacting with several peripheral compartments Drugs entering the systemic circulation require some time to distribute fully throughout the body. In the "postdistributive" phase, where a pseudoequilibrium has been reached between the central compartment (plasma) and the peripheral compartments (other tissues), any changes in the central compartment reflect changes in the peripheral compartments. In this phase, the loss of drug in the central compartment (plasma) or peripheral compartments (other tissues) can be described by a monoexponential process as for the one-compartment model. The half-life for this terminal or postdistributive phase, as for that in the one-compartment, is the time required to eliminate half of the drug from the body.

In the multicompartment model for THC, concentrations in plasma (see figure 7) decrease rapidly, due to distribution of the drug to different tissues. The plasma concentration follows a multiexponential decay, where each disposition phase has a characteristic half-life. As four exponentials are required for the description of the plasma levels of THC, a four-compartment model best

describes the pharmacokinetics of THC (Hunt and Jones 1980). This is indicative of a minimum of four composites of the body compartment into which the drug has variable rates of permeation. The volume of distribution of 600-750 liters, after equilibrium is reached between blood and tissues, is about 200 times that of the plasma volume, indicating that the majority of the drug is accumulated in other tissues. The slow return of THC from sequestered tissues (including adipose or fatty tissue) to plasma is suggested as the reason for the long terminal half-life of THC--about 18 hours. It takes at least 3-4 days to eliminate about 90 percent of THC remaining in the body.

Metabolite Kinetics

The concentrations of metabolites in the body depend on the rate of generation of metabolites from the parent compound and the rate of elimination of the metabolites. After the drug administration, the metabolite concentration will increase until the formation rate of the metabolite is equal to its elimination rate, and then the metabolite level will decrease. When the elimination of the metabolite is very fast, whatever metabolite formed is almost immediately eliminated, and the decline of the metabolite concentration is therefore at the same rate as that of the parent drug. If the elimination rate for metabolite is not significantly faster than the formation rate of the metabolite, the metabolite might build up in the body and decline at a rate slower than that for the parent drug. Tbe elimination half-life in this case is consequently longer than that for the parent drug. The urinary excretion of the metabolite is subsequently a function of how fast the metabolites are formed and excreted.

This latter case is illustrated by a graphic presentation of cocaine and metabolite (benzoylecgonine) levels after the intravenous injection of cocaine, as shown in figure 5. The urinary excretion rates of cocaine and benzoylecgonine are also shown. The terminal half-lives in both plasma and urine for benzoylecgonine are longer than those of cocaine, and consequently the detection of benzoylecgonine after a dose of cocaine is easier than for the parent drug.

Dose-Dependent Kinetics

“Dose-dependent kinetics” refers to a situation where the kinetics of a drug change with the dose administered, an increase in dose resulting in a disproportional increase in plasma concentration. This may be caused by changes in the absorption, distribution, or elimination of a drug with changes in dose. Cocaine, for example, has been reported to be dose-dependent in the dose range of 1 mg/kg to 3 mg/kg (Barnett et al. 1981). The half-life increased from about 40 minutes to 80 minutes, and the clearance decreased from 1.95 liters/hour/kg to 0.6 liters/hour/kg. This means that increasing the administered dose of cocaine 2-3 times may in fact increase the blood concentration (and associated adverse effects) significantly more than 2-3 times.

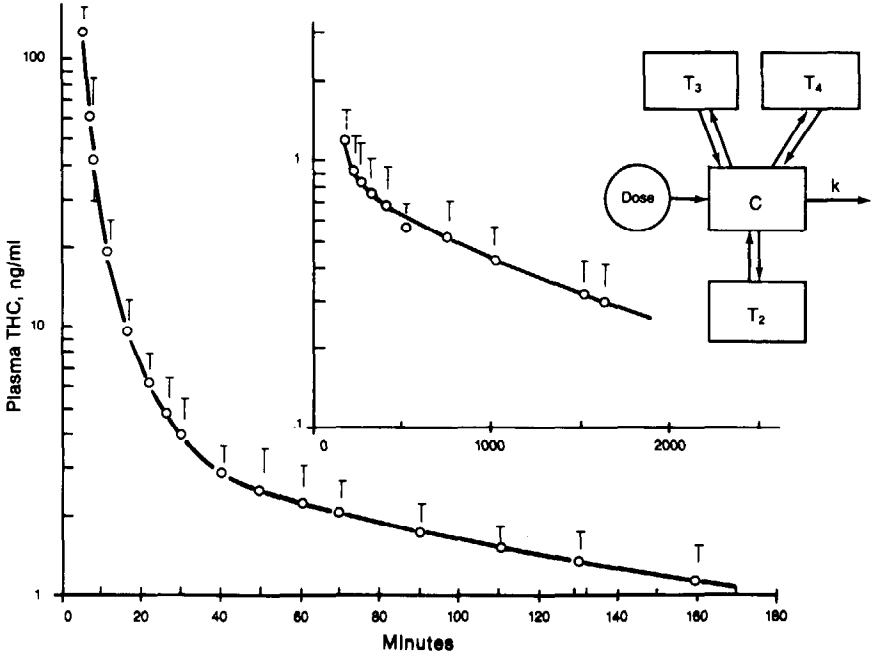

Figure 7. A semilogarithmic plot of average THC plasma concentrations vs. time following a 2-min infusion of 2 mg THC in six subjects. Each point is the group average and each bar represents one standard deviation. The inset is a continuation of the data on a large time scale. (From Hunt and Jones 1980, copyright 1980, American Society for Pharmacology and Experimental Therapeutics). The far right-hand figure is a compartment model consistent with THC plasma kinetics. C is the concentration of THC in the central or plasma compartment; T_2, T_3, and T_4 are the hypothetical tissue compartments.

FACTORS TO BE CONSIDERED IN THE INTERPRETATION OF DRUG CONCENTRATIONS

The interpretation of a drug concentration in biological fluids (blood or urine) to estimate the time of drug administration requires knowledge about the disposition kinetics of the drug. In addition, it requires some knowledge of the route of drug administration, the dose given, and the pattern or frequency of the drug use. This information is very rarely available in a forensic or urine screening case. It is sometimes available in treatment situations when a series of samples over time gives an indication of use patterns along with self-reports (see the preceding chapter).

Disposition Kinetics

The decline of plasma concentrations of a drug depends on the disposition (distribution, metabolism, and elimination) of the drug in the body. The disposition kinetics of a drug for each individual may be different, as each individual may handle the drug differently.

The drug concentration in urine is more variable than that in blood or plasma, as the urine volume and the urinary pH (which affects drug elimination) may change considerably. All the factors that may affect the urine concentration discussed in the previous section on renal excretion have to be considered in the use of urine data. For single samples, the variables involved create a sufficiently great range of possible interpretations to render any specific interpretation questionable other than that drug was probably used in the immediate past (days) by the individual.

Doses

Drug levels in the body depend on the dose given; a higher dose in general produces higher drug concentrations in plasma and urine, etc. Figure 8 shows simulated plasma concentration-time curves of THC when marijuana cigarettes of two different potencies (the high-potency one containing 4 times the THC of the low-potency one) were given to a subject. For the purpose of the illustration, it is assumed that similar kinetics were followed for these two doses, although it has been suggested that a subject may titrate THC intake during smoking (Perez-Reyes 1985). It is seen that the plasma concentration of 2 ng/ml is reached about 1 hour after smoking a low-potency cigarette, while this concentration is not reached until 9 hours after the high-potency cigarette.

It would therefore be very difficult to predict a time of administration from the plasma concentration, even in this idealized situation, if the exact dose were not known. It would be more difficult, if not equal, for the estimation of the time of drug administration without the knowledge of the exact dose.

Route of Administration

As explained in the section on absorption, plasma profiles may be quite different with different routes of administration (see figures 2 and 4).

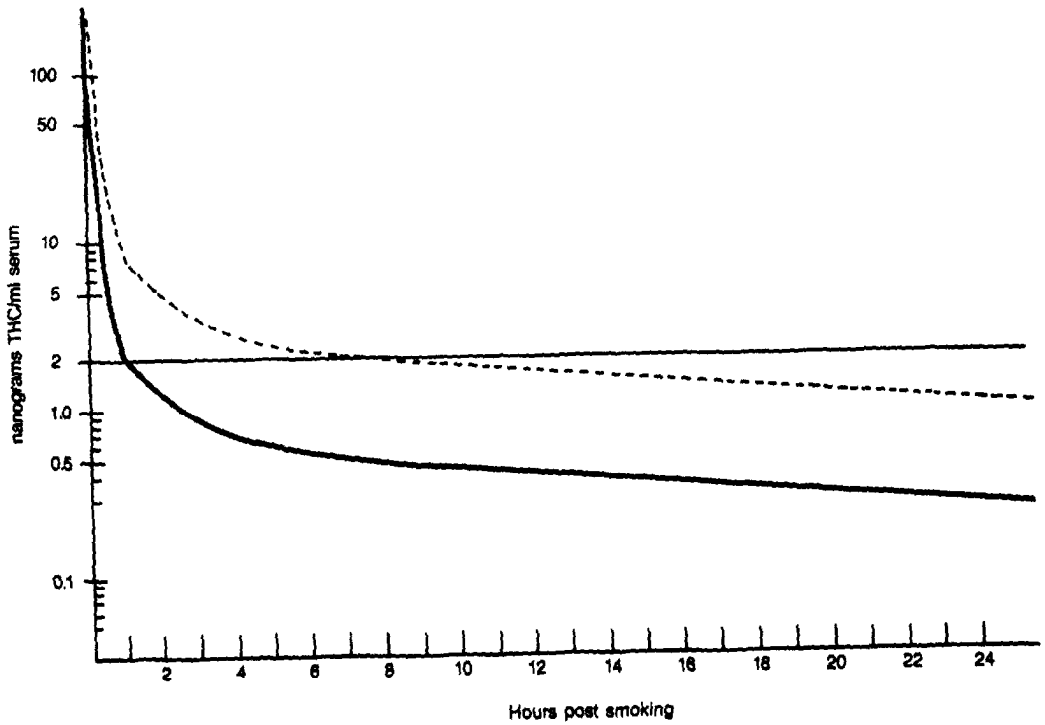

Figure 8. A simulated plasma THC concentrations vs. time curve following smoking marijuana cigarettes of two different potencies of THC content, the high-potency one (broken line) containing 4 times the THC of the low-potency one (solid line), using the pharmacokinetic parameters of THC reported by Hunt and Jones (1980).

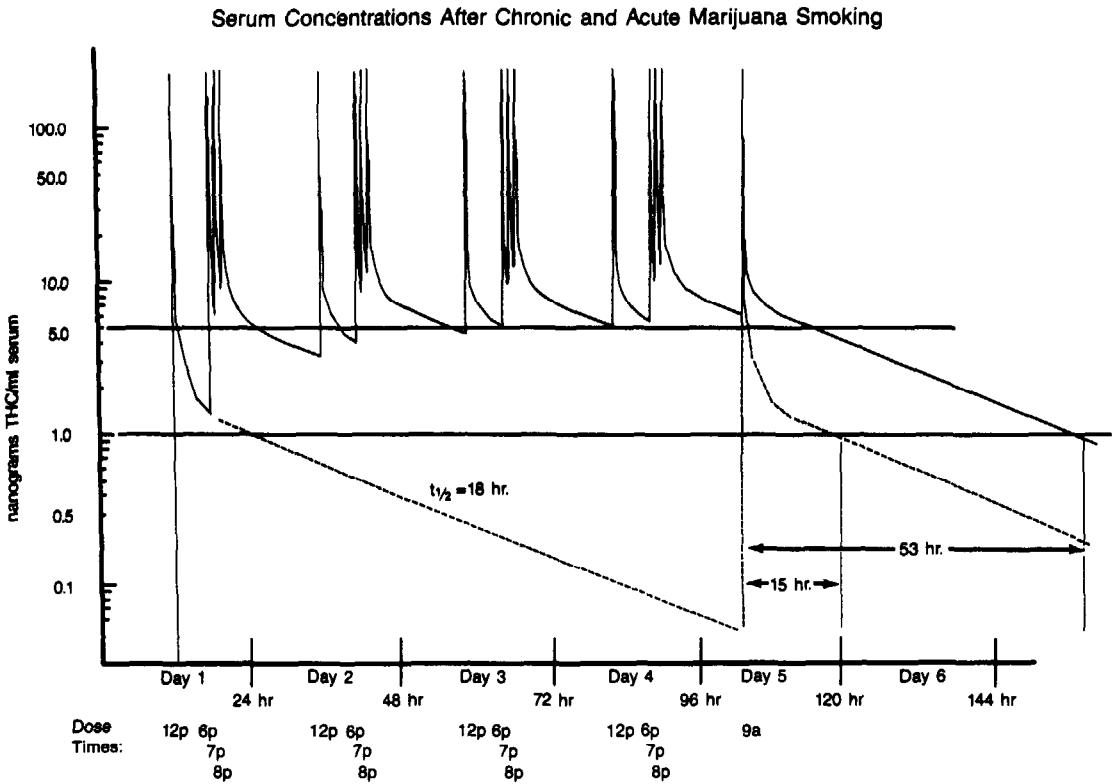

Figure 9. A simulated plasma THC concentration vs. time curve following smoking marijuana at four times a day (at 12, 6, 7, and 8 pm) for 4 days, using the pharmacokinetic parameters reported by Hunt and Jones (1980).

Consequently, the urinary excretion patterns may also be different, although it is difficult to characterize urine excretion patterns as predictive of route of administration.

Chronic Dose vs. Single Dose

When a drug is taken over a period of time, accumulation of the drug or its metabolites may occur if elimination of the drug or metabolites is not complete in the interval between doses. This is illustrated by the simulated curves in figure 9, which compares plasma levels of THC following a single marijuana cigarette (single dose) and four cigarettes a day for 5 days (chronic dose). The THC plasma levels at 24 hours after the last cigarette for the chronic dose is 3 ng/ml, which is about 4 times the level at 24 hours after a single marijuana cigarette. It will take more than 2 days for THC plasma levels to decline to 1 ng/ml after the chronic user (the subject who has taken four cigarettes for 5 days) stops smoking, while it takes about 15 hours for the subject who takes only a single cigarette.

For chronic and heavy marijuana users (cannabis use daily or more often for periods of several months), it has been reported that the marijuana metabolites were detectable in the urine with EMIT-d.a.u. at 20 ng/ml for an average of 31 days with a range of 4-77 days of abstinence (Ellis et al. 1985),

while for light users (cannabis use weekly or less often), it is detectable for an average of 13 days with a range of 3-29 days. Due to the individual variations, substantial overlap occurs in the ranges observed. As explained in the multicompartment model for THC, THC is sequestered in tissues (deep compartment), and the rate of elimination of THC from the body is limited by the slow release of THC from this "tissue compartment." As substantial amounts of THC can be accumulated in tissues for chronic users, considerably long times are required to eliminate it. Consequently, a positive analysis for THC metabolite in urine at these concentration levels may indicate last time marijuana use of a few hours to as much as a few weeks previously.

SUMMARY

Drug concentrations in biological fluids are affected by the dose, route of administration, pattern of drug use, and the dispositional kinetics (distribution, metabolism, and excretion) of the drug. As most drugs are distributed to the site of action by blood, drug concentration measurement in this body fluid provides the best information as to the potential effect on behavior such as driving impairment or on psychological high. Due to wide individual variations in the pharmacokinetics and pharmacodynamics of drugs, however, the use of plasma drug concentrations for the estimation of impairment has not been established for most drugs.

As for urinalysis, drug concentrations in the urine are further complicated by other factors such as urine flow and pH. Even if a specific method is used for the quantitation of a specific drug (the active species, not the inactive metabolite), interpretation in forensic samples to predict time of drug use or impairment is not possible, except within broad time periods, because of the variations in urine drug concentration as well as the limited knowledge available about the dose or the route of administration.

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Examples of Specific Drug Assays

Richard L. Hawks, Ph.D., and C. Nora Chiang, Ph.D.

The following protocols and summary information will illustrate typical combined screening and confirmation techniques for a selected group of abused drugs. Reference to a particular assay or technique does not necessarily indicate a preference for that system. More detailed descriptions of the specific analytical methods mentioned in this chapter can be found in the previous chapter on analytical methodology. For more details about pharmacology, toxicology, and metabolism of drugs, several textbooks on these subjects are available (Baselt 1982, 1984; Goodman and Gilman 1985; Clarke 1986).

The chapter sections are arranged by drug. Each section concludes with a list of references for further information. Drugs discussed are:

- Marijuana/Cannabinoids
- Cocaine
- Amphetamine and Methamphetamine
- Opiates (narcotics)
- Phencyclidine (PCP)
- Alcohol
- Lysergic Acid Diethylamide (LSD)
- Methaqualone
- Barbiturates
- Benzodiazepines

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MARIJUANA/CANNABINOIDS

Delta-9-tetrahydrocannabinol (THC) is the primary psychoactive ingredient present in the leaves and flowering tops of cannabis plants, sold on the street as marijuana. Confiscated marijuana analyzed over the past 10 years shows a steadily increasing potency as defined as the percentage THC by weight found in the plant material. In the mid seventies, this potency averaged about 1 percent. Now it averages 4 percent; a special preparation called sinsemilla averages almost 7 percent. Marijuana is commonly abused by smoking and occasionally by oral ingestion of the plant material.

How Cannabinoids Are Handled by the Body

THC enters the bloodstream rapidly by the smoking route (in minutes) and more slowly by the oral route (1.5-3 hours) and is rapidly transformed by liver enzymes to several metabolites, the primary one being 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid (9-carboxy-THC). THC itself is detectable for a few hours in blood, but because it is so rapidly metabolized and distributed into body tissues, practically no THC is excreted in the urine. THC tends to be stored in fatty tissue and can therefore accumulate faster than it can be eliminated in chronic (repetitive) smokers. This accumulation leads to much longer detection times in urinalysis for the chronic marijuana smoker than for the occasional smoker.

The concentrations of THC metabolites found in urine are influenced by the amount of THC (the dose) absorbed into the bloodstream, by frequency of prior use, by the timing of collection of the urine specimen with respect to the last exposure to marijuana, and by the rate of release of stored cannabinoids from adipose tissue. In addition, the quantity of liquids ingested prior to the time of sampling also affects cannabinoid concentrations in urine (see previous chapters on implications of drug levels and on interpretations of urinalysis results).

Methods of Analysis for Cannabinoids in Urine

Marijuana components and their metabolites have been the subject of many analytical methods. A useful review of recent methods has been published by Cook (in press). Cannabinoids present particularly difficult analytical problems because of their high lipid solubility and low concentrations in body fluids. Lipid solubility increases the difficulty of separating cannabinoids from the biological matrix for analysis.

The immunoassays (EMIT, RIA, and FIA) detect the major metabolite of THC (9-carboxy-THC) in urine, along with probable cross-reactivity to many of the other metabolites of THC and their glucuronide conjugates known to be excreted in urine. These are generally the methods of choice for the initial screening assay. TLC is sometimes used as a screening method, although it is a more labor-intensive approach.

The chromatography methods in current use (GLC, HPLC, TLC, GC/MS) can separate and detect more specifically 9-carboxy-THC itself.

GLC, HPLC, and TLC have all been reported as confirmation methods for 9-carboxy-THC, although they all are subject to interference from co-eluting

substances. Some published methods do not appear to have adequate specificity for use as confirmation methods, while others seem to be adequately validated (see Irving et al. 1984).

Gas chromatography-mass spectrometry (GC/MS) is clearly the most reliable confirmatory method. Both the electron impact (EI) and the chemical ionization (CI) modes have been applied to the quantitation of 9-carboxy-THC. The El spectrum provides generally three ions in a particular ratio characteristic of 9-carboxy-THC, and the CI modes (positive ion CI and negative ion CI) provide a single ion. While the EI spectrum provides more information (three ions), it can be argued that the CI modes are less prone to producing interfering ion fragments and are therefore inherently more specific. The point to be made here is that, regardless of the type of mass spectrometry used, it provides much more specific information and therefore certainty of analysis than other chromatography methods. While other confirmation methods can be adequately validated in some situations, GC/MS will provide the best assurance against legal challenge.

Special Assay Considerations

Acute or occasional (less than twice a week) smoking. Assuming that screening assays of 50 to 100 ng/ml cutoff and confirmation assays for 9-carboxy-THC of about 10-15 ng/ml are used, urine samples will generally be positive for 1 to 3 days.

Chronic (daily) smoking. An individual who smokes regularly even as few as two or three times a week will generally have marijuana-positive urines most of the time. A heavy (daily for months at least), chronic smoker who stops smoking may continue to produce positive samples for longer than a month (depending on the assay cutoff) because of the amount of THC accumulated in the body. It becomes difficult, therefore, to distinguish between the chronic smoker who may in fact have stopped smoking weeks before from the smoker who has not.

Oral administration (ingestion) of marijuana. For the first few hours after a dose, the metabolic profile (relative concentrations of THC and its metabolites) of THC in blood samples, is quite different following a dose taken orally versus the one taken from smoking. However, metabolic profiles in urine samples cannot generally differentiate between a dose taken orally and one taken by smoking.

Passive inhalation of marijuana. Marijuana smoke can be inhaled passively and can result in detectable body fluid levels of THC and 9-carboxy-THC. However, the studies cited generally involve several smokers in a small room or car and one or more nonsmokers in the room for at least an hour with no ventilation. Other studies have used smoking machines to generate the smoke. The probability of this type of exposure leading to a positive urine is, of course, dependent on the sensitivity of the analytical method used, but the screening methods in use today generally incorporate assay cutoffs high enough to make such a possibility highly improbable.

What the Results Mean

A positive urine analysis for THC metabolite(s) indicates that the individual has consumed marijuana or marijuana derivatives within 1 hour to as much as

several weeks before the specimen was collected. While some disagreement exists about what concentration is indicative of "recent" use, it is generally accepted that total cannabinoid concentrations of less than 50 ng/ml by immunoassay in urine may be consistent with usage beyond 36 hours, or from long-term excretion in the chronic user. Without other knowledge of the individual's habits, more specific interpretatiou than this is not usually feasible.

Multiple sampling of urine can frequently help make interpretations more specific. For instance, if several weekly samples are taken from an individual whose fit sample was positive, there should be a different pattern of positives depending on the circumstances of use. If the individual is an occasional or "one time" user, the second or third sample should be negative, along with any subsequent ones. If the individual is a previously heavy chronic smoker who has in fact stopped, samples may be positive for 3 or more weeks, but the concentrations should show a generally decreasing trend, eventually becoming negative for an extended period (2 weeks). If, however, the individual continues to smoke, the samples will continue to be positive for several weeks with no particular indication of a decreasing concentration trend. There has been a recent report by McBurney of a "non-acid" metabolite of THC (8-beta-11-hydroxy-delta-9-THC) which is only detectable in urine for a few hours after the dose. This metabolite may be useful as an indicator of recent use.

A single positive urine test does not mean that the person was under the influence of marijuana at the time the urine specimen was collected. A true-positive urine test means only that the person providing the specimen used marijuana in the recent past, which could be hours, days, or weeks depending on the specific use pattern.

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COCAINE

Cocaine, a central nervous system stimulant prepared from an extract of the coca plant, is self-administered in a variety of ways: snorting, smoking ("freebasing"), and intravenous injection. It is considered one of the most highly reinforcing drugs abused today. Its smoked form (freebase or "crack") appears to be extremely addicting because of the rapid onset and disappearance of its sought-after effect.

How Cocaine Is Handled by the Body

Cocaine is rapidly absorbed (the maximum plasma concentration occurring at about 5 minutes) after smoking. Plasma profiles after smoking are almost equivalent to those following an intravenous dose. There are significant temporal differences in plasma cocaine profiles between the intranasal route and the intravenous or smoking routes. Maximum cocaine concentrations are reached at around 30-40 minutes and persist longer after intranasal inhalation than via intravenous or smoke. The terminal half-life (1.5 hours) of cocaine is the same for all the routes of administration. The fraction of a cocaine dose that enters the circulatory system after the intranasal and smoked routes is estimated at 80 percent and 46 percent, respectively, although large variations occur among individuals.

Cocaine is extensively metabolized, primarily by liver and plasma esterases, and only 1 percent of the dose is excreted in the urine unchanged. Approximately 70 percent of the dose can be recovered in the urine over a period of 3 days. Benzoylecgonine (25-40 percent of the dose) is the major metabolite found in the urine. About 18-22 percent of the dose is excreted as ecgonine methyl ester and 2-3 percent as ecgonine.

Examples of Analytical Methods

lmmunochemical techniques such as EMIT and RIA designed to detect benzoylecgonine are widely used. Unchanged cocaine can sometimes be detected by chromatographic methods for up to 24 hours after a given dose, while benzoylecgonine can generally be detected by immunoassays for 24-48 hours.

Gas-liquid chromatography (GLC), HPLC, and GC/MS are probably the most generally useful techniques for confirmation of cocaine and its metabolite(s) in urine. GC/MS provides the most specific and unchallengeable confirmation.

Special Assay Considerations

Benzoylecgonine can generally be detected in urine up to 2 days after cocaine use. A positive cocaine metabolite assay therefore indicates use within thii period. Depending on the frequency of urine testing, a negative assay may not be a clear indication of lack of chronic use. With this and other drugs that clear the body relatively rapidly, short detection times in urinalysis procedures mean that individuals using such drugs will be less likely to be detected than those using drugs like marijuana or PCP, which have longer detection times.

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AMPHETAMINE AND METHAMPHETAMINE

Amphetamine and methamphetamine (the N-methyl derivative of amphetamine) are central nervous system stimulants usually taken orally, intravenously, or by snorting. Recent reports cite increased use of smoking as a preferred route for methamphetamine by some individuals. Amphetamines increase the heart rate and blood pressure and curb the appetite. In large doses, they cause irritability and anxiety. Tolerance has been observed in amphetamine abusers, and the possibility of developing psychological dependence on the drug is quite significant. Studies have also suggested that chronic abuse may lead to permanent neuronal damage to certain essential nerve structures in the brain.

How Amphetamines Are Handled by the Body

When methamphetamine is administered, some of the drug is metabolized into amphetamine, its major active metaholite, and both of these drugs will appear in the urine. Amphetamine is metabolized to deaminated (hippuric and benzoic acids) and hydroxylated metabolites.

The fraction of a dose of amphetamine excreted unchanged varies with the pH of the urine, with a range of 2 percent (alkaline pH) to 68 percent (acidic pH). In 24 hours, about 79 percent of the dose is excreted in acidic urine and about 45 percent in alkaline urine. Typically, 20-30 percent is excreted as unchanged amphetamine and 25 percent as benzoic acid and its conjugate (hippuric acid).

Methamphetamine is excreted primarily unchanged (44 percent) with a small fraction as amphetamine (6 percent). Its urinary excretion also fluctuates with urinary pH. Under acidic urine conditions, the excretion of unchanged methamphetamine is increased.

Methods of Analysis *for Amphetamines in Urine*

RIA assays such as the Roche Abuscreen will detect only amphetamine, while Syva's EMIT is able to detect both amphetamine and methamphetamine. Although RIA does not react significantly with methamphetamine, sufficient amphetamine is often produced by metabolism to cause a positive response. A more specific Abuscreen amphetamine assay is also available and is sometimes used as a second screen. The new Abbott TDx Drug Detection System is reported to detect both methamphetamine and amphetamine with little or no cross-reactivity to ephedrine and phenylpropanolamine.

Special Assay Considerations

Unchanged amphetamine has been detected in the urine up to 29 hours after a single oral dose of 5 mg amphetamine. Unchanged methamphetamine also has been identified up to 23 hours following a single oral dose.

After chronic intravenous administration, methamphetamine abusers have shown methamphetamine concentrations of 25-300 ug/ml and amphetamine

concentrations of 1-90 ug/ml in urine. Several over-the-counter preparations used as decongestants and diet aids contain ephedrine and phenylpropanolamine, which are capable of producing positive EMIT and RIA tests if present in the urine in significant concentrations. Several prescription drugs, such as benzphetamine, fenfluramine, mephentermine, phenmetrazine, and phenter- mine, can also produce positive immunoassay results.

A positive amphetamine analysis indicates previous use of amphetamine or methamphetamine, generally within the previous 24-48 hours.

Because of the high prevalence of phenylpropanolamine and ephedrine in use for dietary aid and cold remedies and the high probability of their cross-reactivity in immunoassays, it is important to perform careful confirmatory tests for samples screened presumptively positive by immunoassay tests. Gas-liquid chromatography with a nitrogen-phosphorus detector (GLC/NPD) and with appropriate columns for resolving derivatized and underivatized phenylethylamines, and GC/MS with capillary column capability, provide excellent sensitivity and specificity for resolution of presumptive positives by immunoassay tests.

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OPIATES

Morphine, codeine, and semisynthetic derivatives of morphine belong to the class of drugs called opiates. Both morphine and codeine (methylmorphine) are naturally occurring alkaloids from opium, the dried milk juice of the unripe seed pod of the opium poppy Papaver somniferum. An opiate exerts its effects on the central nervous system. Heroin, a semisynthetic derivative (diacetylmorphine) of morphine, is more potent than morphine and is strictly a drug of abuse. Codeine, commonly used in analgesics and cough medicine, and morphine, used in analgesics, are prescription drugs.

Heroin users may take the drug by insufflation (snorting), by subcutaneous injection ("skin-popping"), by intravenous injection ("mainlining"), or by smoking.

How Opiates Are Handled by the Body

Morphine is rapidly absorbed from an oral dose (peak plasma levels at 15-60 minutes) and from intramuscular and subcutaneous injection (peaks at 15 minutes). It is metabolized extensively, with only 2-12 percent excreted as unchanged morphine in the urine. Large amounts (60-80 percent) of the conjugated metabolites (glucuronides) are excreted in the urine, with small amounts (5-14 percent) being excreted in the feces. The quantitatively most important metabolite is morphine-3-glucuronide, which is excreted in the urine to an extent of 67-70 percent of the given dose in 48 hours. The half-life for morphine has been reported in the range of 1.7-4.5 hours.

Heroin is rapidly broken down first to monoacetylmorphine, which is then metabolized to morphine in the body. Both heroin and monoacetylmorphine disappear rapidly from the blood (half-life for heroin is 3 minutes, and that for monoacetylmorphine is somewhat longer), while morphine levels rise slowly, persist longer, and decline slowly. The pattern of urinary excretion of heroin is similar to that of morphine: unchanged morphine (7 percent) and conjugated morphine (glucuronides, 50-60 percent). However, additional trace amounts of 6-acetylmorphine are detectable in the urine.

Codeine is rapidly absorbed from an oral dose; maximum concentrations occur at 1 hour after ingestion. It is extensively metabolized, primarily to conjugated 6-codeine-glucuronide, while 10-15 percent of the dose is demethylated to form morphine and norcodeine, principally in the form of conjugates. Therefore, codeine, norcodeine, and morphine in free and conjugated form appear in the urine after codeine ingestion.

Methods of Analysis for Opiates in Urine

Both EMIT and the Abuscreen RIA detect codeine and morphine in free and conjugated forms but do not distinguish between them. Morphine from heroin use may be detected 2-4 days after the last dose by the immunoassays when these are used at cutoffs of 300 ng/ml. Other narcotics detected by the immunoassays for morphine include dihydrocodeine, dihydromorphine, and hydromorphone. Acid or enzyme hydrolysis of the urine sample is necessary when testing techniques such as TLC, HPLC, and GLC are employed, since approximately 90 percent of codeine and morphine are found in urine in the

conjugated form (glucuronide). Because morphine can come from either heroin or codeine administration, it is important that (1) a confirmatory procedure such as GC/MS, HPLC, or GLC be selected, and (2) the sample be hydrolyzed prior to analysis.

Special Assay Considerations

A screening assay that is positive for opiates could be the result of several different circumstances of drug administration. Since immunoassays do not distinguish between codeine, morphine, or their glucuronide conjugates, a confirmation test that is specific for morphine and/or codeine is necessary. The presence of morphine alone or its conjugate can indicate either clinical morphine use or illicit morphine or heroin use (within the previous 1-2 days). The presence of both morphine and codeine in urine is consistent with ingestion of codeine alone, when the codeine concentration is high and greater than that of morphine, which can be produced as a metabolite in urine. Prescribed use of codeine must be ascertained in this case. Generally, ingestion of a therapeutic dose of codeine (30 mg) will lead to detectable levels of the free morphine or codeine for only a few hours, although other metabolites may be detectable for 2-3 days by immunoassay.

Street heroin also contains acetylcodeine, which metabolizes to codeine. Therefore, in cases of low morphine and codeine concentrations in urine, it is not possible to determine whether the subject has taken heroin, codeine, or morphine. Although codeine presence in urine may indicate illicit drug use, its presence in many antitussive or analgesic prescription preparations makes such a conclusion questionable.

The ingestion of a large quantity of poppy seeds can result in positive opiate findings in urine samples by immunoassay methods up to 60 hours after ingestion. This is apparently due to trace amounts of morphine in commercial poppy seeds. A recent report by Fehn and Megges suggests that an analysis by GC/MS for 6-O-acetylmorphine, a metabolite of heroin can be used to distinguish between an opiate positive urine resulting from poppy seed ingestion versus heroin use (1985).

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PHENCYCLIDINE

Phencyclidine (PCP, or angel dust) is commonly taken orally, by inhalation (smoked), by insufflation, or intravenously. The drug can be added to parsley, mint, oregano, or other leaves and smoked in the form of a cigarette. In liquified form, it can be swallowed, injected, or applied to smoking material. Several PCP derivatives produce similar pharmacological effects.

How PCP Is Handled by the Body

Phencyclidine is well absorbed following all routes of administration. Maximum plasma PCP concentrations are observed 5-15 minutes after smoking. In one study, approximately 40 percent of PCP spiked on a cigarette entered into smoker's bloodstream as PCP and 30 percent as phencyclohexene, a decomposition product of PCP. Oral absorption of PCP is slower; the maximum plasma concentration is observed at 2 hours after the dose. About 72 percent of the oral dose is absorbed. The terminal half-life for PCP varies considerably, with a range of 8-55 hours and average of 18 hours.

PCP undergoes oxidation and conjugation in the body. Unchanged PCP is excreted in the urine in moderate amounts (10 percent of the dose). Metabolites identified are primarily conjugates of hydroxylated PCP. About 40 percent of the material in the urine has not been identified. Excretion of PCP is increased in acidic urine, but excretion of polar metabolites is unaffected by urinary pH. PCP may be detectable in urine for several days to several weeks. Some PCP is secreted in the saliva.

Examples of Analytical Methods

Immunochemical methods are relatively specific for PCP, its metabolites, and some of the closely related analogs. Methods for confirmation include gas-liquid chromatography with nitrogen-phosphorus detection (GLC/NPD), with differential columns to rule out interference from other drugs, or gas chromatography-mass spectrometry (GC/MS), which will provide the most specific and unchallengeable assay for PCP and its analogs.

Special Assay Considerations

A positive urine assay for PCP generally indicates drug use within the previous week. There have been reports that sufficient PCP can be absorbed through skin to lead to a positive urinalysis. The use of saliva has been reported to correlate well with blood as a means of detection of recent PCP use. Hair analysis for the detection of PCP has also been reported.

False positives in immunochemical assays for PCP have been reported with the administration of thioridazine (Mellaril), dextromethorphan, and chlorpromazine (Thorazine). This supports the need for specific confirmation of the screening analysis.

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ALCOHOL

With the possible exception of caffeine, ethyl alcohol is the most widely used drug in our society. The determination of alcohol in bodily fluids is perhaps the most commonly performed drug analysis.

How Alcohol Is Handled by the Body

Alcohol is used orally, and its concentration in the blood at any given time is influenced by such factors as a person's weight, the rate at which the individual drinks, whether food is taken with the "dose," and tolerance. Blood alcohol concentrations (BACs) are usually expressed as "percent" (g/deciliter) or as "mg percent" (mg/dl). Drinking 1.5 oz of 86 proof alcohol in a short period of time will result in a BAC of approximately 0.03 percent (or 30 mg percent) in a 160-pound individual.

The liver metabolizes 95 percent of the ingested alcohol at a relatively constant rate. A normal liver will metabolize alcohol at approximately .015 g/dl per hour. This means it takes the body about 2 hours to metabolize 3/4 oz of pure alcohol. This is equivalent to a 5-oz glass of wine, a 12-oz can of beer, or a 1.5 oz glass of 88 proof liquor. If a person drinks at a rate faster than 1.5 oz per hour, the blood concentration (and the effects) will accumulate.

If a 160-pound individual took four drinks each containing 1.5 oz of 100 proof liquor within 4 hours, a BAC of about 0.08 percent (80 mg percent) would be reached. The same number of drinks or a six-pack of beer imbibed over a l-hour lunch period might render the individual above the legal limit for the rest of the workday.

Examples of Analytical Methods

Methods for analysis are classified as chemical, biochemical, and gas chromatographic. Chemical methods utilizing acid potassium dichromate solutions are not specific for ethyl alcohol but can be used to provide rapid screening for volatile substances. If negative, no further testing is required. If positive, a confirmation test is necessary to ensure the presence of ethyl alcohol.

Biochemical methods for alcohol analysis employ an enzyme. The enzyme alcohol dehydrogenase (ADH) is combined with other reagents in a kit form and is available from several manufacturers. The enzyme method is reliable and accurate when used by experienced analysts. The enzyme is fairly specific in that it does not react with methanol or acetone, but it does react to some degree with isopropyl and long chain alcohols. Although these higher molecular weight alcohols are rarely encountered, positive identification for ethyl alcohol is required in legal situations.

Gas chromatographic methods offer excellent proof for the identification of ethyl alcohol and are the most widely used. These methods can simultaneously detect other alcohols, ketones (acetone from diabetics), and other volatile substances. Of the three types of methods, gas chromatographic procedures are the methods of choice.

A variety of techniques involve extraction, distillation, or direct injection. All are reliable when an internal standard is incorporated into the procedure for quantitation purposes. More recently, automated head space analyses are being used; they offer excellent precision and accuracy, in addition to providing rapid turnaround time.

What the Results Mean

Unlike most of the other drugs discussed in this monograph, alcohol is a legal drug and its presence in urine does not indicate illicit activity. An alcohol analysis is always aimed at determining the BAC in order to relate this to a particular level of impairment or at least to a legally defined definition of impairment. In practice, evidence or suspicion of alcohol abuse is best confirmed by breath or blood analyses to determine the concentration present. If this concentration is more than (or close to) the legally accepted presumptive concentration for impairment (100 mg/dl or 0.1 percent in most States), grounds exist for an assumption of impairment.

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LYSERGIC ACID DIETHYLAMIDE

Lysergic acid diethylamide (LSD) is an extremely potent drug capable of producing altered mental states at doses as low as 25 micrograms (approximately one-millionth of an ounce). The drug is relatively easily prepared from natural products. Although LSD was most widely used during the 1960s. it continues to be available through illicit channels, and there is evidence that its use is again increasing. The drug is not physically addictive, and there are no known. examples of deaths directly attributable to the pharmacological effects of LSD. While under the influence of the drug, however, a person's ability to perform complex mental and physical tasks is severely affected. Furthermore, use of the drug is known to sometimes cause bizarre behavior, which can lead to fatal accidents and suicides.

How LSD Is Handled by the Body

Following oral ingestion of LSD, the effects are perceived within a few minutes and usually last for about 12 hours. Recurrence of the drug's effects long after its use ("flashbacks") has been reported, but the actual cause of this phenomenon is not understood. Studies with laboratory animals have shown that LSD is rapidly metabolized and only a small proportion of the dose is excreted in the urine as the parent drug. Few reports have been published regarding the concentrations of LSD and its metabolites in urine following ingestion of the drug by man. However, because only small amounts of LSD are ingested and because it is likely that the drug is rapidly metabolized in man just as it is in laboratory animals, concentrations of the drug in the urine of a user are unlikely to exceed a few nanograms per milliliter. In a recent study, after administration of LSD to two volunteers at a dose of 1 microgram of drug per kilogram of body weight, the concentration of the parent drug in the urine reached a maximum of 1 to 2 ng/ml between 2 and 8 hours, and then decreased to less than 0.1 ng/ml within 20 hours. A radioimmunoassay (RIA) with a cutoff of 0.5 ng/ml for LSD-reactive substances gave a positive test for the urine collected from the same subjects for up to 30 hours after administration. Two metabolites of LSD could be detected by a highly sensitive GC/MS assay in the urine specimens collected for up to 72 hours after administration.

Methods for Analysis of LSD in Urine

Most published assays for LSD are intended for identification of the drug in illicit preparations and do not offer the sensitivity and specificity required for detection of LSD in urine specimens from users. A radioimmunoassay for LSD was recently introduced commercially by Roche Diagnostic Systems, Nutley, NJ, and appears to offer an effective means of detecting very recent use of the drug. However, confirmation of the presence of LSD or its metabolites remains a difficult task. High-performance liquid chromatography combined with fluorescence detection has been used to detect LSD at urinary concentrations as low as 0.5 ng/ml. A method employing the combination of capillary column gas chromatography and electron ionization mass spectrometry has been developed and is also capable of measuring LSD concentrations as low as 0.5 ng/ml. However, neither of these assays is useful for detection of LSD in urine for more than about 12 hours after ingestion. A GC/MS assay for the N-demethyl and 13-hydroxy metabolites of LSD in urine has been developed and shown to permit

detection of LSD use for more than 2 days after ingestion. Other analytical techniques that have been evaluated for detection of LSD in urine include the combination of liquid chromatography and mass spectrometry (LC/MS) (Kidwell, personal communication) and tandem analyzer mass spectrometry (MS/MS).

Special Assay Considerations

The task of developing an assay with sufficient sensitivity to detect LSD or its metabolites in urine from users is made more difficult by the drug's sensitivity to both light and heat. However, two studies have shown that LSD is stable in urine for more than a month when stored at or below room temperature and protected from direct sunlight or other sources of ultraviolet radiation. LSD analyses based upon gas chromatography require a well-deactivated capillary column in order to avoid severe adsorptive loss of the drug. Conversion of LSD to its N-trimethylsilyl or N-trifluoroacetyl derivative results in improved gas chromatographic behavior and permits higher sensitivities to be achieved.

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METHAQUALONE

Methaqualone (Quaalude) is a sedative-hypnotic drug taken orally in doses of 75-300 mg. Far higher doses may be taken in nontherapeutic abuse situations. Chronic use in tolerant individuals of over 3 g per day has been reported.

How Methaqualone Is Handled by the Body

The absorption of methaqualone is rapid, with peak plasma concentration reached in 1.5-2 hours. The drug is extensively metabolized in man; less than 1 percent of the dose is excreted as unchanged methaqualone in the urine. Approximately 30 percent of the dose is excreted in the urine in 24 hours, 25 percent as hydroxylated metabolites (12 have been identified).

After the ingestion of a therapeutic dose (300 mg) of methaqualone, methaqualone is negligible (usually less than 1 ng/ml) in the urine, while metabolites may still be detectable for more than a week by sensitive GC/MS methods.

Examples of Analytical Methods

EMIT and RIA not only detect unchanged drug but can significantly detect various metabolites of the drug, thus providing longer detection periods after last ingestion. Confirmatory methods for the detection of unchanged methaqualone and conjugated (bound) metabolites after hydrolysis of the sample include GLC and GC/MS.

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BARBITURATES

Barbiturates are central nervous system depressants and used as hypnotics/sedatives. They are classified as ultra-short, short-, intermediate-, and long-acting. The duration of action of barbiturates is quite variable, ranging from 15 minutes for ultra-short-acting drugs to a day or more for long-acting drugs. The most commonly abused barbiturates are short- and intermediate-acting agents such as pentobarbital (Nembutal), secobarbital (Seconal), and amobarbital (Amytal). Long-acting agents such as phenobarbital are rarely subject to abuse.

How Barbiturates Are Handled by the Body

Barbiturate derivatives are excreted into the urine in varying amounts of unchanged drug and metabolites. Long-acting barbiturates like phenobarbital are excreted with a higher percentage of unchanged drug in the urine, while short-acting barbiturates, secobarbital and amobarbital, are extensively metabolized and excreted in the urine with a smaller percentage of unchanged drugs.

Examples of Analytical Methods

EMIT and RIA are designed to detect unchanged secobarbital in the urine; however, both assays will detect other commonly encountered barbiturates, depending on the concentration of drug present in the sample. Phenobarbital positives have been noted in chronic users up to several weeks after cessation of use. With standard single doses of secobarbital, pentobarbital, or amobarbital, RIA and GLC identified drug presenee for up to 52 hours and 76 hours, respectively, utilizing a 100 ng/ml cutoff. TLC, less sensitive than RIA, demonstrated the presence of barbiturate for approximately 30 hours under the same conditions. With high dosages and/or chronic daily doses, EMIT and RIA can be used effectively for screening. Gas-liquid chromatography/flame ionization (GLC/FID) after derivatization, GLC/NPD and GC/MS are reliable methods used for confirmation of the various barbiturates.

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BENZODIAZEPINES

The benzodiazepines are considered by many as the most prescribed drugs in the United States. They are primarily used as antianxiety and sedative-hypnotic drugs and also have broad therapeutic use as anticonvulsants and muscle relaxants. The benzodiazepines have a low order of acute and chronic toxicity when used in a medically supervised manner. Chronic use does result in some moderate dependence and tolerance to the drug. More than a dozen benzodiazepines are in clinical use today. The best known benzodiazepine drugs are Valium (diazepam) and Librium (chlordiazepoxide).

How Benzodiazepines Are Handled by the Body

These drugs are well absorbed when administered orally, the most common route of administration. Most benzodiazepines are extensively metabolized in the liver and excreted in the urine as metabolites. Nonconjugated metabolites may possess pharmacological activity that may account for the "next day" effects for some benzodiazepines. Many of the new benzodiazepines are metabolites and derivatives of the old drugs. For example, oxazepam is a common urinary metabolite of many benzodiazepines and is also a marketed drug (Serax).

The duration of action and elimination half-lives of the different benzodiazepines vary widely. The half-lives for major benzodiazepines are: chlordiazepoxide, 5-10 hours; diazepam, 30-60 hours; oxazepam (Setax), 5-10 hours; flurazepam (Dalmane), 2-3 hours for the parent drug and 50-100 hours for active metabolites.

Because of the long elimination time for the benzodiazepines, an individual who has been using a drug for months or years may maintain detectable urinary concentrations of the drug for weeks to months after discontinuation of its use.

Examples of Analytical Methods

A broad spectrum of analytical methods, GC, HPLC, TLC, GLC/MS, RIA, and EMIT, has been reported for the analysis of the benzodiazepines, or their metabolites, or of their acid hydrolysis products, e.g., benzophenones. Because many benzodiazepines have common metabolites, it is not always possible to determine the drug taken through the use of urine testing. The EMIT screening procedure is rapid and specific for benzodiazepiues, as it detects thii class of drugs by recognizing oxazepam, a common metabolite of many benzodiazepines, and also many cross-reacting benzodiazepine drugs or metabolites. Other screening procedures used to detect benzodiazepines alone or in combination with major drugs of abuse in urine screening programs can be achieved by TLC and HPLC. Radioimmunoassay methods possess the necessary sensitivity for the determination of diazepam directly in microsamples of blood/plasma (10 ul) and saliva (100 ul) for up to 16 hours following oral administration of a single 5 mg dose of the drug.

Confirmation of positive results from tests performed with immunoassay or thin-layer chromatography, which detect many of the metabolites, may be

difficult using more specific techniques such as GLC and GC/MS. Care must be taken to ensure that the metabolites detected with TLC or immunoassay will chromatograph when using GLC and GUMS techniques.

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Glossary

Accuracy--Ability to get the correct (or true) result.

Analyte--Substance to be measured.

Blank--Biological specimen with no detectable drugs added, routinely analyzed to ensure that no false-positive results are obtained.

Blind sample--Control material submitted to the analyst (unknown to him or her) as a routine specimen.

Chain of custody--Handling samples in a way that supports legal testimony to prove that the sample integrity and identification of the sample have not been violated, as well as the documentation describing these procedures.

Concentration--Amount of a drug in a unit volume of biological fluid, expressed as weight/volume. Urine concentrations are usually expressed either as nanograms per milliliter (ng/ml), as micrograms per milliliter (ug/ml), or milligrams per liter (mg/l). (There are 28,000,000 micrograms in an ounce, and 1,000 nanograms in a microgram.)

Confirmation--A second test by an alternate chemical method to positively identify a drug or metabolite. Confirmations are carried out on presumptive positives from initial screens.

Creatinine--An endogenous substance appearing in the urine.

Cross-reacting substances--In immunoassays, refers to substances that react with antiserum produced specifically for other substances.

Cutoff level (threshold)--Value serving as an administrative breakpoint (or cutoff point) for labeling a urine result positive or negative.

Detection limit--Lowest concentration of a drug that can reliably be detected.

False negative--An erroneous result in an assay that indicates the absence of a drug that is actually present.

False positive--An erroneous result in an assay that indicates the presence of a drug that is actually not present.

Interfering substances--Substances other than the analyte that give a similar analytical response or alter the analytical result.

Metabolite--A compound produced from chemical changes of a drug in the body.

Pharmacokinetics--The study of the time course of the processes (absorption, distribution, metabolism, and excretion) a drug undergoes in the body.

Pharmacodynamics--The study of the relationship of drug concentration to drug effects.

Precision--Ability to get the same result between repeated measurements.

Presumptive positive--Sample which has been flagged as positive by screening but which has not been confirmed by an equally sensitive alternative chemical method.

Proficiency-testing specimen--A specimen whose expected results are unknown to anyone in the laboratory, known only by an external agency, and later revealed to the laboratory as an aid to laboratory improvement and/or a condition of licensure.

Quality Assurance (QA)--Practices that assure accurate laboratory results.

Quality Control (QC)--Those techniques used to monitor errors which can cause a deterioration in the quality of laboratory results. Control material most often refers to a specimen, the expected results of which are known to the analyst, that is routinely analyzed to ensure that the expected results are obtained.

Qualitative test--Chemical analysis to identify the components of a mixture.

Quantitative test--Chemical analysis to determine the amounts or proportions of a mixture.

Screen--A series of initial tests designed to separate samples with drugs at the particular minimum concentration from those below that minimum concentration (positive versus negative).

Sensitivity--The detection limit, expressed as a concentration of the analyte in the specimen.

Specificity--Quality of an analytical technique that tends to exclude all substances but the analyte from affecting the result.

Split specimen--Laboratory specimen that is divided and submitted to the analyst, unknown to him or her, as two different specimens with different identifications.

Standard--Authentic sample of the analyte of known purity, or a solution of the analyte of a known concentration.

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