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# TESTING FOR DRUGS OF ABUSE: METHODS AND RELIABILITY

Edward A. Kaufman, M.D.\*

## FOREWARD

Testing for drugs of abuse has become one of the most heated topics of our times. Testing advocates cite safety hazards and the loss of tens of billions of dollars in decreased productivity as a result of drug abuse on the job. Opponents are concerned about the challenge to individual rights implicit in some forms of testing, and much has been said and written about the risks and consequences of improperly conducted tests. The role of a responsible clinical laboratory is neither to advocate nor to discourage the development of drug testing programs, but rather to provide clear information on the available procedures and their limitations, to conduct those tests with impeccable attention to accuracy and confidentiality, and to back up positive results with flawless medico-legal documentation and expert witness testimony.

The sudden expansion of demand for drugs-of-abuse testing has given rise to a growing number of hastily improvised testing services and new specialty laboratories, and the questionable quality of these operations has produced a great deal of bad publicity and costly litigation. Yet testing for substances of abuse as performed at a reputable, well-established laboratory is extremely accurate and reliable. A positive result obtained from such a laboratory and backed by meticulous chain-of-custody documentation can be accepted with virtual certainty as evidence of drug use.

Providing this degree of quality and reliability is no easy matter, however. The laboratory must be ever alert to the many technical and medico-legal factors that can affect the viability of a test result. Although most of the news media's ongoing debate about accuracy has focused on the testing process itself, there are many steps that precede and follow the actual testing that require an equal degree of care.

The purpose of this paper is to discuss the entire testing process, from specimen collection and preparation (vital processes that are often beyond the control of the laboratory) to test methods, quality assurance measures, medico-legal documentation, and result reporting and interpretation. Each of these steps demands careful attention, and the hallmark of a responsible, quality-conscious laboratory is that it can offer a complete testing system that focuses not only on the assays themselves but also on each of the necessary precautions and transactions so crucial to the viability of test results.

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## CHOOSING THE TYPE OF SPECIMEN

### *Urine*

In most cases, urine is the specimen of choice for drug testing. Urine collection is easy and noninvasive. Drugs are readily detected in urine specimens, since this is the primary route by which drugs and their metabolites are excreted from the body.

### *Blood*

Analysis of blood specimens is useful for establishing the time of drug use, since most drugs are quickly metabolized in blood and rapidly disappear from detection. In addition, blood is collected by means of a "closed system," which greatly reduces the possibility of contamination of the sample. However, blood specimens must be obtained under sterile conditions by a trained technician, and the procedure for drawing blood is necessarily invasive. Furthermore, the rapid disappearance of drugs from blood makes their detection technically more difficult than in urine.

## PREPARATION OF THE URINE SPECIMEN

### *Collection*

The optimal method of collection is to have the participant urinate into a collection container under observation. The specimen is then transferred to one or two (optional) specimen bottles.

### *Checking Specimen Viability*

As a check on specimen viability, the pH and specific gravity should be determined using a residual specimen from the original collection container (optional). These determinations should be confirmed by the laboratory before testing begins. A fresh, unadulterated specimen will have a pH between 5 and 8 and a specific gravity greater than 1.005. It has also been suggested that the temperature of the specimen be measured immediately after collection. A reading approximating body temperature offers some degree of assurance that another specimen has not been substituted for the original.

### *Labeling*

Each specimen bottle must bear a clearly legible name and/or identification code.

*Tamper-Evident Tape*

Specimen bottles are carefully sealed with tamper-evident tape to enable laboratory technologists to recognize tampering and request a repeat specimen.

*The Requisition Form*

A requisition form should be submitted stating the name and/or identification code of the participant and the test to be performed.

*Chain-of-Custody Documentation*

The chain of custody must be clearly documented to make sure that test results will be accepted as legal evidence..Chain-of-custody forms should be signed or initialed at each transfer of the specimen's custody.

*Sealed Chain-of-Custody Bags*

The taped specimen bottles, requisition form, and chain-of-custody form should be sealed in a tamper-evident chain-of-custody bag before the specimen leaves the site of collection.

## TRANSPORTING THE SPECIMEN

A well-established delivery system using laboratory-employed couriers is an important element in maintaining specimen integrity and reliable chain-of-custody documentation. Laboratory couriers are specially trained in proper handling of specimens and in chain-of-custody requirements, ensuring fast and legally supportable delivery of specimens to testing sites. Although licensed commercial carriers and the United States mail can also provide chain-of-custody documentation, use of laboratory couriers allows an added degree of control over transportation procedures and delivery times.

## TESTING: A TWO-TIERED PROCESS

A responsible laboratory will issue a positive test result only if the drug or drugs in question have been detected by two distinct tests using chemically different analytic methods. The most practical means of fulfilling this re-

quirement is to perform an initial *screening* test on all samples and then to follow up with a *confirmatory* test on all samples that have been found to be positive on the initial screen. If a sample is determined to be negative on the screening test, or if the screening results are positive but the confirmatory results are negative, the result reported for each drug is "None Detected." Only if both the screening test *and* the confirmatory test are positive is a report issued that a specific drug has been "Detected."

### *The Initial Screen*

Screening can be defined as testing urine samples of completely unknown composition specimens to determine the presence or absence of drugs and, if present, their probable identity. The principle requirement of a screening method is that it be highly sensitive. That is, a screening test should be very sensitive to the presence of the substance for which the test is being conducted. However, tests with a high degree of sensitivity tend to be less reliable in establishing a positive identity of the substance detected, and the very sensitivity that makes the screen so useful can also result in misidentification of chemically similar compounds. For example, some screening tests for amphetamines cross-react with phenylpropanolamine, a commonly used decongestant. This limited accuracy of screening tests has provided the news media ample opportunity for questioning the reliability of drugs-of-abuse testing as a whole, and indeed, *screening tests should never be used without automatic confirmatory testing of positive results by a more specific method.*

Screening assays fall into two basic categories: immunologic assays and thin-layer chromatography procedures.

*Immunoassays.* The present consensus seems to be that immunologic assays are the methods of choice for screening. One of the most widely used assays is the enzyme-multiplied immunoassay technique, which is performed using a kit manufactured by the Syva Company (a division of Syntex, Inc., located in Palo Alto, California) under the trade name "EMIT." The EMIT assay is similar in principle to other commonly used immunoassays, including the enzyme-linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA).

The EMIT system can be used to detect approximately 35 different drugs of abuse. In screening for common drugs of abuse, up to 10 separate EMIT assays may be performed, using 10 different sets of reagents. The more common drugs that can be detected with the EMIT screen include:

*Amphetamines*  
 d-Amphetamine  
 Methamphetamine

*Barbiturates*  
 Amobarbital  
 Butobarbital  
 Pentobarbital  
 Phenobarbital

*Benzodiazepines*  
 Chlordiazepoxide (Librium)  
 Clonazepam (Clonopin)  
 Diazepam (Valium)  
 Flurazepam (Dalmane)  
 Oxazepam (Serax)

*Opiates*  
 Codeine  
 Heroin (detected as morphine)  
 Hydrocodone  
 Hydromorphone  
 Morphine

*Other Drugs*  
 Cannabinoids (marijuana metabolites)  
 Cocaine (detected as benzoylecgonine)  
 Methadone  
 Methaqualone (Quaalude)  
 Phencyclidine (PCP)  
 Propoxyphene (Darvon)

The EMIT assay is performed by following a complex sequence of steps. First a drop of urine is mixed with antibodies against the drug being sought, known quantities of the drug labeled with an enzyme, and a substrate on which the enzyme can act. The unlabeled drug in the specimen and the added enzyme-labeled drug compete for binding with the antibodies. As a result, the more unlabeled drug there is in the specimen, the more enzyme-labeled drug is left free to react with the substrate. The enzyme-substrate reaction causes a color change that is measured with a spectrophotometer. The degree of color change represented by this measurement is proportional to the amount of drug and/or drug metabolites present in the original specimen.

*Thin-Layer Chromatography (TLC).* Thin-layer chromatography is a traditional analytic method that can be used to screen for a broader range of drugs (as many as fifty) than the immunologic assays. TLC is a useful, sensitive technique, but it is more labor-intensive and is significantly more dependent on the analytic skill of the laboratory technologist than EMIT and other automated immunoassays. This method is most often applied in clinical settings, especially when the cause of coma or suspected drug overdose must be determined.

#### *Confirmatory Testing*

Although confirmatory techniques are as sensitive as screening techniques, the primary criterion of confirmatory methods is that they be highly

specific. That is, they must be very accurate at determining the *absence* of the substance identified as present by the screen if in fact that substance is not present in the specimen. Confirmatory tests should be performed on a separate sample taken from the original specimen bottle (or, if two bottles are submitted, taken from the second bottle) to eliminate possible errors in the screening test. Commonly used confirmatory methods include high-performance thin-layer chromatography (HPTLC), gas chromatography (GC), and gas chromatography in conjunction with mass spectrometry (GC/MS).

*High-Performance Thin-Layer Chromatography (HPTLC).* In thin-layer chromatography (TLC), an extract of urine is placed at the bottom of a thin glass plate coated with grains of silica. The edge of the glass plate is then placed in a solvent, which migrates up the plate, carrying the drug along with it. Different drugs migrate different distances, depending on their physical and chemical properties. The resulting drug deposits are invisible and need to be "developed," much like photographic film. Identification of the drug is based on the shape and color of these deposits as well as the distance they travel.

In HPTLC, a finer, more uniform silica is used. This enhances separation and permits more accurate drug identification. HPTLC is suitable as a confirmatory test for certain drugs.

*Gas Chromatography (GC).* More technically known as gas-liquid chromatography, GC is initiated by injecting a small quantity of processed urine residue into a chromatographic column -- a coiled glass tube lined with inert liquid silicone, or an equivalent, and ranging in length from a few feet to hundreds of yards. Some gas chromatographs contain two columns with slight variations in retention characteristics, thus providing duplicate results. Immediately following injection, the sample is subjected to high heat and vaporized. This vapor is then pushed through the tube by pressurized neutral gas.

The gasified molecules of different drugs move through the tube at different speeds, according to their individual chemical and physical properties. At the far end of the column, the molecules enter a device called a detector. This device breaks the molecules into electrically charged fragments called ions. These ions create a current when they strike the detector, thus providing a means of measuring the time that has elapsed between sample injection and detection. Since each drug moves through the column at its own unique speed, these transit times, referred to in the laboratory as retention times, are compared to known standard retention times for each of the drugs suspected of being in the sample.

Dual-column GC is considered by toxicologists to be one of the most reliable forms of confirmation for amphetamines, cocaine, benzoylecgonine (the cocaine metabolite found in humans), PCP, and a number of other drugs of abuse.

*Gas Chromatography/Mass Spectrometry (GC/MS).* GC/MS is a highly specific analytical technique whereby vaporized drugs pass from the gas chromatograph into a special detection device known as a mass spectrometer. In the mass spectrometer, the gasified molecules are bombarded and shattered by a stream of electrons. Each drug has its own unique pattern of ions, which can be identified on the basis of number and mass. These ions are directed through a "gate," the size of which is progressively increased to admit ions of successively greater mass, to reach the detector where the ions create a measurable current.

The mass spectrometer requires only a fraction of a second to measure the full range of ion fragment sizes in the sample. The resulting data are organized by a computer and displayed in a graph that shows the mass of the various ion fragments and the number of ions of each mass. This unique ionic pattern provides a "molecular fingerprint" that allows identification of the drug through comparison with a computerized library of known molecular fragmentation patterns.

GC/MS is considered one of the most accurate analytical methods for identifying drugs in body fluids and is especially helpful in identifying marijuana and its metabolites (cannabinoids).

## QUALITY ASSURANCE

Daily quality assurance procedures are essential in maintaining result reliability. Quality assurance measures in a reputable laboratory should include frequent analysis of standard and control specimens to monitor and recalibrate analytic instruments, regular proficiency testing to maintain licensure and accreditation, and blind submission of control samples as a means of evaluating every step of the testing process.

### *Standard and Control Samples*

Analytic instruments must be painstakingly recalibrated every day by analyzing standard samples containing known quantities of drugs. Readings for successive standards must be consistent. This process is essential for ensuring the accuracy of test results.

Following the initial instrument calibration, control samples containing known amounts of drug should be run with each batch of specimens to make sure the instrument retains its accuracy. When results for the control samples are inaccurate, all of the specimens in the batch must be retested. At

SmithKline Bio-Science Laboratories, controls are run before and after each batch and following every tenth sample.

### *Proficiency Testing*

To maintain licensure and certification, laboratories must undergo regular proficiency testing. This testing requires the laboratory to analyze samples submitted by state departments of health and such private groups as the American Association for Clinical Chemistry and the College of American Pathologists. The types of testing required for the samples is specified by the testing agency, but the laboratory is not told which substances may or may not be present or in what concentrations. Test results are reported to the agencies, which base their accreditation on the attainment of set levels of proficiency.

### *Blind Submission of Control Samples*

Blind proficiency testing is an internal quality check performed by only the very best laboratories; there are no commercially available blind testing programs. This type of proficiency testing is very similar to the testing described in the previous paragraph. Outside clients are asked to mix blind samples with their own specimens. Laboratory personnel have no way of knowing which samples in a batch, if any, are quality control samples. In this way, the entire testing system can be evaluated, from courier pick-up to reporting of results. Blind proficiency testing is the only real way of making sure that a laboratory is giving the necessary attention not only to the analytical procedures themselves, but to all the many contingent factors on which accuracy and reliability depend.

## TEST ACCURACY

"Accuracy" can be defined in a variety of different (and evasive) ways, but the practical essence of the term can be found in a question asked by every conscientious client: "What is the probability that a drug is really present in the specimen when the test result is positive?" A responsible answer to this question can be given only after performing a series of calculations to obtain what is called the predictive value of a positive test. The predictive value must be calculated on the basis of three statistical measures: sensitivity, specificity, and prevalence. Many claims have been issued about the sensitivity and specificity of various tests, but none of these three values, quoted alone or in combination, should be confused with accuracy.

### *Prevalence (Prev.)*

Prevalence is a measure of the frequency with which an event occurs in the population being studied. In substance abuse testing, prevalence is the fre-

quency with which one would expect to encounter drug users among a population of employees prior to testing. These data are derived from a company's experience and may be based on prior testing for that organization, testing conducted for other institutions, or information provided by other employers with similar employee profiles. A prevalence of 15% indicates that 15 of every 100 employees can be expected to be drug users.

#### *Sensitivity (Sens.)*

Sensitivity represents the ability to detect a given substance with a test or combination of tests. The sensitivity states the probability that known positive samples will actually test positive. For example, if a test identifies 95 of every 100 known positive samples as positive, then that test has a sensitivity of 95%.

#### *Specificity (Spec.)*

Specificity represents the ability to declare a sample negative when it is in fact negative for a given substance. The specificity states the probability that known negative samples will actually test negative. For example, if a test identifies 90 of every 100 known negative samples as negative, then that test has a specificity of 90%.

#### *Predictive Value (PV)*

The predictive value, or the probability that a positive test result has in fact identified a true drug user, is calculated on the basis of a complex revision of the foregoing three values using Bayes theorem:

$$PV = \frac{\text{Prev.} \times \text{Sens.}}{[\text{Prev.} \times \text{Sens.}] + [(1-\text{Prev.}) \times (1-\text{Spec.})]}$$

Using the examples given earlier:

$$\begin{aligned} \text{Prevalence} &= 0.15 \\ \text{Sensitivity} &= 0.95 \\ \text{Specificity} &= 0.90 \end{aligned}$$

$$PV = \frac{0.15 \times 0.95}{[0.15 \times 0.95] + [0.85 \times 0.10]} = 0.63$$

This means that if a positive result is obtained from the screening test, there is a 63% probability that the test has correctly identified a drug user.

Obviously, then, a sensitivity of 0.95 and a specificity of 0.90 do not necessarily mean that the test is at least 90% accurate. This example can be considered representative of an actual screening test. A drug use prevalence of 15% is somewhat greater than average, but a screening test with a sensitivity of 95% and a specificity of 90% would be considered a good test. Yet a positive result could hardly be considered conclusive evidence of drug use.

This clearly illustrates why it is essential to follow every positive screening test with a confirmatory test. The confirmatory methods discussed in this paper are highly specific, and a positive confirmatory test in conjunction with a positive screening test raises the probability that the drug has been correctly identified to more than 99.99%. That is, the predictive value in such a case exceeds 99.99%. Thus the uncertainty is virtually eliminated, and the result is highly reliable.

## INTERPRETING TEST RESULTS

### *What Does a Positive Result Mean?*

A confirmed positive result offers nearly 100% assurance that the specimen tested does in fact contain a drug or drugs. However, assays for drugs of abuse do not indicate how the drugs were used, nor can they distinguish between drugs that were obtained legally and drugs obtained or used illegally. Equally important is the fact that tests for drugs of abuse detected in urine can provide only a very general and unreliable estimation of the time of drug use, and a positive test result gives no indication of whether or not the user's behavior was affected at the time of specimen collection.

### *Importance of the Medical History*

Because these tests do not distinguish between drugs used legally and those used illegally, it is important to obtain a record of relevant medical information before test results are interpreted. The need to obtain this information may pose a challenge to medical confidentiality, and this issue must be resolved by the employer, the employee, and the employee's physician. This is one of several reasons that it is important to use testing for drugs of abuse only as part of a more comprehensive program.

### *Test Reliability*

Again, a confirmed positive test result obtained by a reputable, quality-conscious laboratory can be considered highly reliable as an indicator of actual drug use.

RESULT REPORTING, SPECIMEN STORAGE,  
AND  
EXPERT TESTIMONY

*Reporting*

Most laboratories issue their results either by teleprinter or on computerized report forms delivered by company couriers. Maintaining confidentiality is important for both ethical and legal reasons, and these reports should be treated with the same care accorded medical reports.

In most cases, negative results are reported as "None Detected," and the substances for which the laboratory was asked to test are listed. Positive results are usually reported with the word "Detected" next to the substance or substances identified in the sample.

*Specimen Storage*

Specimens should be stored by the laboratory for one to two weeks to allow for retesting to settle any questions that may arise. In some cases, specimens are sent to another laboratory for additional confirmatory testing. If necessary, positive specimens can be stored for as long as two years to allow for confirmation of test results in case of later disputes.

*Expert Testimony*

A conscientious laboratory will be willing to back up test results with expert testimony by qualified staff toxicologists and pathologists. Testimony may be required on a variety of relevant issues, including the chain-of-custody, test accuracy and reliability, interpretation of test results, quality assurance programs, and laboratory methods, materials, and procedures.

CONCLUSION

As performed by a reputable laboratory, testing for drugs of abuse is both highly accurate and reliable. However, this reliability is dependent on the proper performance of a wide variety of functions. The laboratory must monitor the entire testing system, from specimen pick-up to reporting of results. Careful use of sophisticated equipment is in itself insufficient to provide reliable and legally viable results.

In choosing a laboratory, each client company must first assess its own needs and then evaluate how those needs match the capabilities of the laboratories under consideration. Important factors in choosing a laboratory include accuracy, service, turnaround time, and range of testing. Companies

must also learn proper techniques for specimen collection and preparation, since these critical functions are usually beyond the control of the laboratory.

In summary, drug screening tests offer an efficient means of excluding negative specimens from further consideration. Specimens that produce positive results on this initial screen must then be retested by an appropriate, chemically different confirmatory method to eliminate any false positives. If this procedure is followed by an established, quality-conscious laboratory, the accuracy of a confirmed positive result should approach 100%. However, it is important to be aware of the limitations of drugs-of-abuse testing when interpreting test results. Because of these limitations, such testing is best implemented as just one important element of a more comprehensive response to employee drug abuse.

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