overview [chemistry]

Urine Drug Testing: Approaches to Screening and Confirmation Testing

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- Most drugs of abuse in urine (DAU) testing have relatively short windows of detection periods (up to 3 days) except for those with high lipid solubility (benzodiazepines, up to 21 days; cannabinoids, up to 60 days).
- Immunoassays have high sensitivity for DAU screening but lower specificity because of cross-reactivity with substances other than the drugs in question.
- It is important to establish patterns of drug usage and populations of interest because they may have an impact on which DAU to include in screening panels.
- Confirmation of DAU testing is the use of a second analytical method to positively identify a drug or metabolite in urine.

Approximately 24 million urine drug tests were performed annually in the United States at a cost of about $1.2 billion per year, according to a 1994 study by the Committee on Drug Use in the Workplace of the National Research Council and the Institute of Medicine and commissioned by the National Institute on Drug Abuse.[1] That number would be expected to be even higher now, 10 years later. The service of urine drug testing is usually provided by the clinical laboratory. The laboratory must make many crucial decisions with regard to the drugs to test, including the tests to be included in panels, the analytical methodologies employed, the protocols for screening and confirmation testing, and the types of drug testing to be offered. This review is intended to provide the clinical laboratory with an overview of the factors which influence these decisions and the options available to the laboratory.

Drugs of Abuse in Urine

Drug abuse is usually defined as the excessive and persistent use, usually by self-administration, of any drug, licit or illicit, which may lead to adverse physical or psychological consequences. Drug abuse is not always associated with any degree of frequency but is often associated with drug use in quantities which may result in some degree of toxicity in the user and which may reflect a pattern of psychopathological behavior.[1,2] In fact, there is a continuum from minimal use to abuse to addictive drug use.[3] Most drugs of abuse are regulated by the government, primarily by the schedules of the Controlled Substances Act.[2] Examples of drugs of abuse include depressants (opioids, barbiturates, benzodiazepines, alcohol), stimulants (amphetamines, cocaine), hallucinogens (LSD, mescaline, phencyclidine), and cannabinoids (marijuana).

Sample Choice, Length of Urine Drug Detection

The laboratory plays a central role in the detection of drugs of abuse in human urine specimens. Urine is the preferred specimen for drug testing primarily because it is non-invasive (in contrast to drawing a blood specimen, an invasive procedure which may require patient consent). Drug levels in blood only reflect presence of drug at a given point in time, and levels may be high enough to be detected only for a relatively short period of time. Urine specimens may contain detectable levels of drug over an extended period and at much higher concentrations than in blood. Urine may also contain higher levels of drug metabolites than blood, providing further evidence of drug use. **T1** outlines the advantages and disadvantages of urine specimens for DAU testing.[2]

Urine Drug Testing, Advantages and Disadvantages **T1**

Advantages
• Non-invasive
• Ample volume
• Drugs and drug metabolites found in urine are usually stable
• Drugs and their metabolites are often present in higher concentrations in urine than in other biological materials
• Detectable in urine for relatively long period of time
• Presence of metabolites (in addition to parent drug) provides further evidence of drug use
• Readily preserved by refrigeration or freezing
• Analysis relatively simple because of absence of proteins and cellular material in urine
• Wide availability of commercial reagents and analytical systems

Disadvantages
• Drug levels in urine do not correlate well with levels in other body fluids
• Drug levels may vary widely depending on fluid intake, voiding pattern, and time lapse since drug intake
• Urine drug excretion continues after physiologic effect of the drug ceases, resulting in lack of correlation of drug level with intoxication
• May be difficult to obtain specimen if test subject cannot void (catherization?)
• Urine specimens are easily substituted, diluted, or adulterated
• Direct observation may be an invasion of privacy
• Urine may be unstable if not properly handled and stored

The window of detection for drugs of abuse in urine may be very variable and may be affected by many factors, some of which are shown in **T2**. **T3** displays some approximate detection times of some common drugs of abuse in urine. Drugs with unusually high lipid solubility (benzodiazepines and cannabinoids) are often detectable in urine for weeks, in contrast to other DAU, some of which are detectable only for a few days.

Specimen Integrity, Substitution, Adulteration/Dilution

The collection of urine samples for DAU testing must include a process to ensure the integrity of the urine specimen. Every effort must be made to minimize the opportunity for the test subject to substitute, adulterate, or dilute the collected specimen prior to drug testing.[4] Direct observation collection (DOT) of the urine specimen collection procedure offers maximum assurance of specimen integrity, but because of opposition to witnessed collection and individual privacy, DOT may not always be appropriate, unless there is a reason to suspect that the test subject may substitute or alter the urine specimen.[4] Federal guidelines list the following circumstances which would create a reasonable belief of substitution or adulteration[4]:

1. Urine specimen outside normal body temperature range (32.5°C-37.7°C, 90.5°F-99.8°F).
2. Individual's last provided urine specimen had a specific gravity <1.003 and creatinine concentration <0.2 g/L.
3. Test subject clearly and unequivocally acting in a manner indicating an attempt to substitute or adulterate the specimen.
4. Follow-up or return-to-work test being collected under DOT rules for an individual previously verified to have a positive DOT test.

Laboratories may also perform a urine pH determination to verify that the urine specimen pH is within the limits established for healthy human subjects (pH 4.5 to 8.0). After urine specimen collection, a documented procedure for the handling and transportation of the urine specimen, ideally using chain of custody procedures, should be followed.

The test subject may attempt to substitute his/her specimen with a "drug-free" urine, or with water, synthetic urine, or other fluids such as soda, tea, or apple juice during the urine collection process.[4] Commercially available synthetic urines purported to enable the user to pass drug tests are sold on the Internet.

The test subject may attempt to tamper with urine specimen in vivo by the intentional ingestion of a substance in an effort to affect the drug test result. Most commonly, the subject may consume large quantities of water in an attempt to increase urine output, thereby reducing the drug of abuse concentration to a level below the cutoff concentration for that drug.[4] Likewise, ingestion of diuretics (either pharmaceutical or foodstuffs) will give rise to a more dilute urine. Ingestion of foods which affect urinary pH may also alter the urinary excretion of a drug; for example, ingestion of soda crackers will cause an alkalinization of urine which affects the renal excretion of methadone, resulting in a negative finding for a patient currently receiving methadone.[5]

The test subject may attempt to adulterate a urine specimen in vitro by the addition of substances to the urine specimen in an effort to affect the drug test result. Various chemicals and household agents such as salt, bleach, liquid Drano, soap, detergents, vinegar, lemon juice, tea, salt, and eye drops have been used by drugs addicts to invalidate the results of enzyme and fluorescent polarization analyses for DAU.[6-9]

Analytical Procedures, Cutoff Concentrations, False Positives

By definition, DAU screening tests are a series of initial tests designed to separate urine samples with drug levels at or above a particular minimum concentration (the cutoff concentration for a positive screening test) from those containing no drug or with levels below that minimum concentration (negative screening test). Screening assays have been approved for commercial use as in vitro diagnostic test by the Food and Drug Administration. In general, the analytical techniques used for DAU screening are primarily immunoassays which use antibodies to detect the presence of a drug or metabolite in urine. In this technique, a known amount of antibody and labeled drug or metabolite (antigen) is added to the urine specimen. The amount of labeled antigen able to bind with the antibody is a function of the urine concentration of

Some Factors Which Affect Urine Drug Levels **T2**

Time and size of last dose
Single dose versus multiple doses
Volume of fluid intake prior to collection
Kidney function (excretion of drug)
Liver function (metabolism of drug)
Kinetics of drug distribution including volume of distribution, lipid solubility, urinary half-life

Approximate Detection Times Of Some Common Drugs Of Abuse In Urine **T3**

Drugs	Duration of Detection in Urine
Alcohol	up to 1 day
Amphetamines (including MDMA, MDA)	1-2 days
Barbiturates	1-3 days
Benzodiazepines	Up to 21 days
Cannabinoids	Up to 60 days*
Cocaine	1-3 days
Methadone	1-3 days
Opiates (including codeine and morphine)	1-3 days
Propoxyphene	1-3 days

**At 50 ng/mL cutoff concentration*

the drug or metabolite present. Spectrophotometric endpoints, which may be enzymatic or fluorescent for these reactions, are used to identify drugs and/or metabolites in urine. In a recent College of American Pathologists (CAP) survey of laboratories enrolled in the CAP urine drug screening program (UDS), of the approximately 2,850 laboratories reporting results for cocaine and metabolites, the CAP breakdown on laboratory methods showed that about 44% of laboratories used an enzyme immunoassay technique, 39% of laboratories used a fluorescent immunoassay, and 12% used a point-of-care assay.[10]

The initial screening DAU assay is used by the laboratory to eliminate urine specimens which are below the cutoff concentration for that drug or drug class. The cutoff concentration, used to separate negative specimens from specimens which require further testing, will vary according to the screening technique used, the manufacturers' recommendations, and the laboratory's policy. Usually, the cutoff concentration will be the concentration of the lowest calibrator provided by the manufacturer. **T4** provides cutoff concentrations for the enzyme multiple immunoassay technique (EMIT, Syva, Dade Behring) for some common DAU assays.

Immunoassays are widely used by clinical laboratories in the United States because they are inexpensive, rapid, easily automated, and have high sensitivity for detection of DAU. The major weakness in the immunoassay screening techniques for DAU is the less-than-perfect specificity for their designated drug(s) of abuse. There may be cross-reactivity due to the presence of substances other than the analyte(s) of interest (false-positive result). The degree of cross-reactivity with substances other than the drug of interest will vary from assay to assay. Also, there may be variable cross-reactivity to a parent drug and its metabolites, thus making it extremely difficult to determine all possible substances which may cause a false-positive reaction. For example, a metabolite of ranitidine crossreacts with the immunoassay for amphetamines, causing a false-positive result even though therapeutic levels of the parent give a negative response.[11,12] False positive results for amphetamines may also be caused by a number of sympathomimetic drugs including ephedrine, pseudoephedrine, and phenylpropanolamine, which may be present in over-the-counter cold and decongestant medications.[13]

False-positive results for common opiate screening assays are encountered in patients receiving antibiotics of the quinolone class that crossreact with the opiate immunoassay.[14] Poppy-seed food products, such as poppy-seed bagels or muffins, may cause a true-positive immunoassay screening test for opiates due to the presence of low, but still detectible, levels of opium alkaloids.[15] A screening cutoff of 2,000 ng of morphine is often used to eliminate positives due strictly to poppy seed ingestion; however, a substantial number of positives due to true opiate abuse will also be missed at this high cut-off level.

Patterns of Drugs of Abuse Usage and Drug Screening Panels

Drugs-of-abuse usage may differ significantly depending on the population of interest [ie, age, environment (urban versus rural), geographical areas (United States versus other countries), etc].[16-18] For example, methamphetamine abuse is much more common on the West Coast than the East Coast, although the drug is slowly spreading eastward.[18]

It is important to determine drugs-of-abuse usage patterns in a particular population, since it may have a significant impact on use and costs for DAU screening testing. Many laboratories have set up DAU panels for clinician convenience and to facilitate the ordering of DAU screening. However, the widespread use of DAU panels may have unintentionally led to over-utilization and unnecessary drug testing, especially given the ease of ordering a panel with many DAU drugs when perhaps a much smaller panel of fewer DAU would suffice.[19] In a recent study, the positive screening rate for 8 DAU screening tests was determined, resulting in a proposal to standardize the DAU panel to 3 DAU tests, excluding DAU tests with very low positive screening rate or a DAU test needed specifically for methadone. The study showed that after the adoption of a standard DAU panel, there was a 47% decrease in DAU utilization and costs.[19]

Common Cutoff Concentrations For DAU Screening Testing (EMIT) **T4**

Assay	Cutoff Concentration (ng/mL)
Amphetamine/ Methamphetamine	1,000
Barbiturate	200
Benzodiazepine	200
Cannabinoids	50, 100
Cocaine Metabolite	300
Methadone	300
Opiates	300, 2000
Propoxyphene	300

DAU Confirmation Testing and Analytical Procedures

Confirmation of DAU testing is defined as a second test by an alternate chemical method to positively identify a drug or metabolite. Confirmation testing is carried out on presumptive positive specimens as determined by the initial immunoassay screens.[20] The selection of the analytical method for confirming the presence or absence of drugs or metabolites in a specimen will depend on the purpose of the DAU testing. If drug-testing results are used as a guide in medical diagnosis or in patient therapy and do not affect the test subject adversely, it may be unnecessary to confirm in order to establish unequivocally the presence of the initially positive DAU. In cases where further testing is desired, a second presumptive determination involving a repeat of the immunoassay screening may be all that is required.[2] It should be emphasized that the performance of the second presumptive determination is not a substitute for confirmatory testing but is designed to reduce operator errors that can cause false-positive results. When a repeat of the assay is not sufficient, a second, confirmatory,

assay, using a different chemical or physical principle, would be utilized.

Confirmatory DAU testing may be done in situations where employment status, reputation, or freedom of the patient may be in question or when laboratory results are needed for legal/evidentiary purposes.[20] The analytical techniques used for drug confirmation include chromatographic techniques such as high performance liquid chromatography and gas chromatography. The analytical technique established as the "gold standard" for confirmation of drugs of abuse in urine is gas chromatography/mass spectrometry (GC/MS) because it provides the most accurate and unequivocal results for drug identification possible.[20,21] The GC/MS combines 2 different analytical processes. GC physically separates the various drugs and metabolites following their extraction from a urine specimen, while MS provides a positive identification of the individual components separated by GC. The GC/MS usually involves the initial application of a solid phase or solvent-solvent extraction procedure to isolate and concentrate the drugs of interest from the urine sample. Following reconstitution, the extract is injected into the GC/MS for the separation, identification, and quantification of the individual drugs or metabolites. The GC/MS is an expensive procedure requiring sophisticated instrumentation, a high level of technical expertise, and a knowledgeable experienced operator who can interpret the GC/MS data. In a recent urine forensic drug CAP survey, there were only 128 laboratories who participated in the forensic drug identification program in the United States.[22]

When to Confirm a Positive DAU Screening Test

F1 shows an algorithm for DAU testing. If the result for the DAU screening test is negative for the DAU tested, the urine specimen is by definition negative and no further testing needs to be done. However, the reader needs to be aware that in DAU testing, a negative result should always be interpreted to mean that the laboratory was "unable to detect the presence" of the drugs of interest. The sample may be truly negative, or the sample may contain drug at a concentration below the cutoff level for a positive finding. If the DAU screen is positive for a drug, the need for and type of confirmation testing will depend on the purposes for testing and the populations tested.

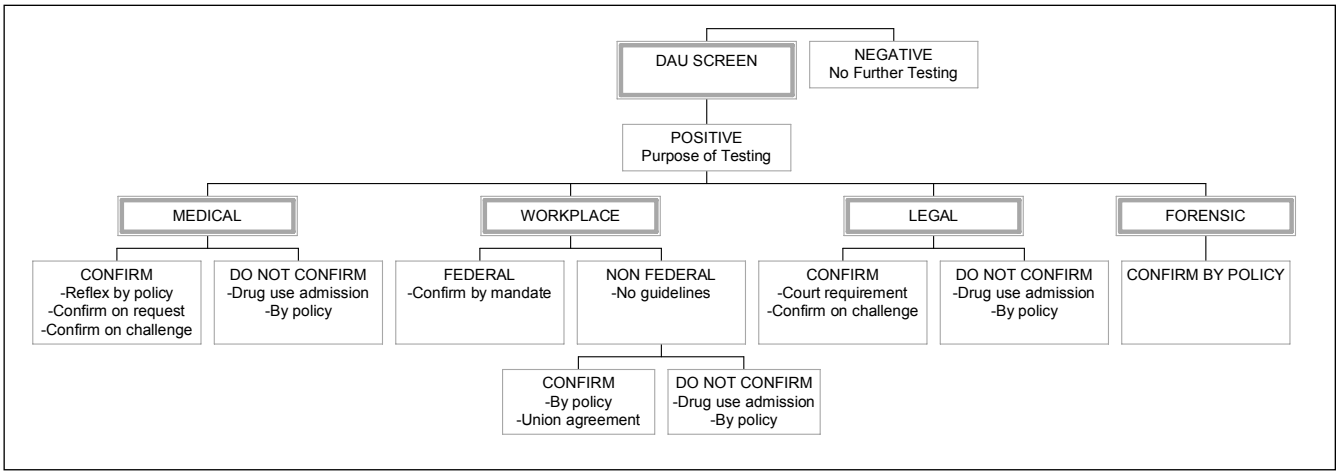
Medical DAU Testing

Drugs-of-abuse testing for medical purposes involves testing patients in drug programs (including drug rehabilitation programs) or who are suspected to be abusers of controlled substances both to verify drug dependency and to monitor treatment progress.[2] Medical purposes for DAU testing also include compliance testing (is the patient taking or not taking a prescribed and potentially abuseable drug) and overdose testing (is the patient's impairment a result of overdosing on an abusable drug).

Confirmation in the medical setting may be performed in a reflex fashion, which requires a screened positive DAU must be confirmed by administrative policy. A positive DAU screen test may also be confirmed by physician request, especially if the physician is suspicious of the DAU result. A positive DAU screen may also need confirmation if the patient challenges the DAU screening result and adamantly denies drug abuse. When DAU confirmation is not done, it is usually due to administrative policy or because the patient admits to drug use.

Workplace DAU Testing

Drugs-of-abuse testing in a workplace situation involves testing of populations which must be initially drug-free or who need to remain drug-free because drug use may present a security and/or safety risk in the workplace.[21] Typically, pre-employment drug testing is used to screen applicants for substance abuse problems. Workplace testing for DAU may also be performed randomly on employees who must remain drug free in jobs involving safety risks such as airline pilots, train operators, or bus drivers.[21] Testing is also done for cause, when there is reasonable suspicion of



[**F1**] Algorithm for DAU testing.

drug abuse, or following an on-the-job accident, or other unsafe act.

When President Ronald Reagan signed Executive Order 12564 on September 15, 1986, the stage was set for a drug-free federal workplace. The guidelines, established for mandatory drug testing throughout the executive branch of the federal government, applied to all executive departments, the uniformed services, the intelligence community, and to all other employing units or authorities of the federal government, except the United States Postal Service, the Postal Rate Commission, and employing units in the judicial and legislative branches.[23] Subsequent federal regulations extended testing into specific industries such as transportation and nuclear energy. The Office of Workplace Initiatives of the National Institute on Drug Abuse (NIDA) sought to promote private sector companies to institute workplace drug testing.[23]

Federal guidelines for workplace drug testing programs mandate a confirmation test by GC/MS if the initial immunoassay screening test is positive, as well as an interview with a medical review officer to ensure that another substance which the worker may be taking legitimately is not causing a false-positive result.[4,21] There are no guidelines for non-federal workplace drug testing. Confirmation of DAU may be by administrative policy or by union agreement designed to protect the right of the employee. There may also be no DAU confirmation because of drug use admission by the prospective employee or by employer policy. Often, private employers may choose not to confirm the initially positive screening test for pre-employment drug testing, since the cost of confirmatory GC/MS may be 5 to 6 times the cost of the screening test. Furthermore, there is no assurance that the private employer will adhere to the recommendation that the applicant be informed of the initially positive screening result and be given a chance to challenge the DAU positive finding—the applicant may never know why he was not hired, the employee may never know the true reason he was laid off.[24]

Workplace drug screening is a very big business, with 1994 drug testing cost estimates of at least $1.2 billion in the United States based on about 24 million drug tests performed annually, 18 million in Substance Abuse and Mental Health Services Administration (SAMHSA) certified laboratories, and 6 million by non-certified laboratories[24,25]; half of major United States companies now randomly test their employees, and more than 500 school districts have urine drug screening programs.[24] It is further expected that, with the emphasis being placed on the testing of students by the present administration, there will be an even greater increase in workplace testing.

Legal Purposes for DAU Testing

The purpose of drugs-of-abuse testing for legal reasons is to provide reliable information to the courts regarding an individual's use or nonuse of an abuseable drug. Drug testing for legal purposes must meet strict criteria for sample integrity, chain-of-custody documentation, and confirmation of drug identification by a reference method of drug analysis. The GC/MS is the preferred method of confirmation, as there is a sizable body of medical literature and legal precedents supporting its validity. Testing for DAU in the legal setting may be done because it is required by the court, or it may be done *a priori* because the employer fears a legal challenge to an action he may take against an employee as a result of a positive drug screen.

Forensic Purposes for DAU Testing

The purpose of drugs-of-abuse testing for forensic reasons is to provide a sensitive, specific, and conclusive identification of all drugs present. By definition, forensic testing is used for the purpose of criminal investigation, and therefore it must meet strict criteria for sample integrity, chain of custody documentation, and confirmation of drug identification by a reference method of drug analysis in much the same way as legal testing. Thus, for forensic purposes, confirmation is performed by policy. Forensic testing is much more complex than legal testing, (discussed above) as many body fluids and tissues are examined, not just urine. Additionally, in forensic testing, it is wise to identify all compounds, including drug metabolites and adulterants that also may be present.

Economic Impact of DAU Confirmation Testing

In the present era of shrinking health care resources and emphasis on cost-effective laboratory utilization, health care organizations and clinical laboratories are challenged by the need to control costs. One way of controlling costs is to develop and implement a confirmation policy for initially positive-screened immunoassay results based purely on economic considerations. An approach designed to reduce the demand and cost of DAU confirmation would be to alter laboratory protocols that automatically require confirmation of an initially positive DAU screening test and to adopt a policy for confirmation by clinician request only. This approach would be most acceptable in patient drug rehabilitation and treatment programs in which the primary purpose of DAU screening and testing is to verify drug use and to monitor drug treatment progress. A recent study found that there were 5,331 confirmation tests done when the laboratory required confirmation of an initially positive DAU screening test, but after adoption of a confirmation by clinician-request-only protocol, there was a 95% decrease in the number and related costs of DAU confirmation tests performed by the laboratory.[19]

Another approach would be 1 outlined by the United States Department of Justice, Bureau of Justice Assistance, which has advanced an interesting option for parole and probation programs. In their paradigm, if a drug screen is positive and the parolee challenges the findings, a confirmation test would be done with the parolee paying the testing costs if the screening result confirms positive.[26]

Conclusion

Approaches to the screening and confirmation testing of drugs of abuse in urine involve a complex interplay of laboratory, medical, and legal factors which may influence decisions. The choices of drugs to be screened, the composition of testing panels, the intended purpose and use of the drug testing results, the need or desire for confirmation testing, and the economic costs of testing are all factors that must be considered by the laboratory. This review is intended to assist the laboratory as a guide for DAU testing and in the selection of appropriate screening and confirmation testing within the context of the individual laboratory's resources, medical needs, and budget.

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