National Laboratory Certification Program
DRUG TESTING MATTERS

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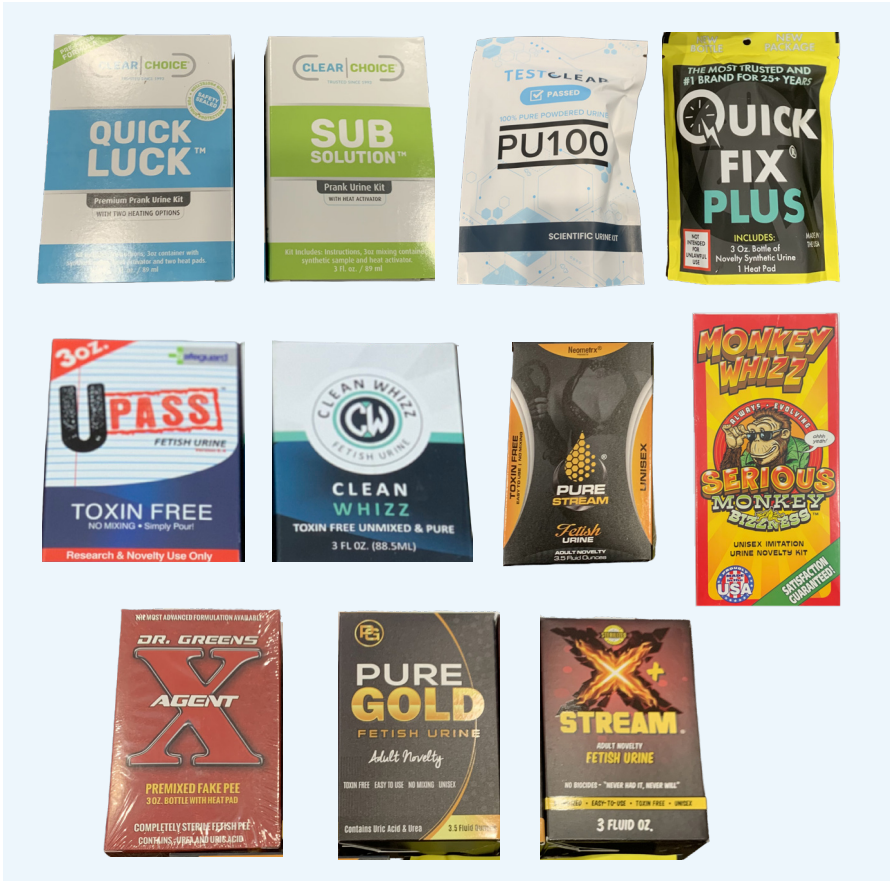
An Introduction to Specimen Validity Testing in Workplace Drug Testing

Specimen validity testing (SVT) refers to testing used to detect subversion through adulteration or dilution of authentic urine specimens or through the substitution of the specimen with synthetic urine. These tests are used in combination with collection protocols to mitigate subversion and ensure federal workplaces remain drug-free. Initial urine SVT includes screening for pH, creatinine, and the presence of oxidants. No SVT is currently required for oral fluid specimens because all collections are observed.



The first iteration of the Mandatory Guidelines for Federal Workplace Drug Testing Programs using Urine (UrMG) was published in 1988 after the Drug-Free Workplace Program was created. However, it was not until April 2004 that the UrMG[1] were updated to include requirements for specimen validity testing (SVT) in response to subversion attempts by donors.

Drug Test Subversion Strategies

Drug test subversion, or "beating the test," is probably as old as drug testing itself. Drug test subversion strategies can approximately be divided into three categories: adulteration, substitution, and detoxing. Adulteration refers to adding something to the urine specimen to mask the analytes or interfere with testing. Substitution refers to replacing the urine specimen with another liquid, such as someone else's urine or a product made to mimic human urine. The last category is best described as detoxing, and includes behavioral changes before the collection, such as consuming large quantities of water, to reduce drug concentrations in the matrix.

Previously, the most common strategy of subversion was adulteration; however, because of improvements in SVT, a recent review of online discussions[2] about drug test subversion showed that substitution, such as replacing the urine specimen with a clean specimen (either synthetic urine or somebody else's urine), is currently most common. Detoxing, such as consuming large quantities of water to dilute the urine specimen, was also discussed, but adulteration was rarely mentioned.

The discussion below about of SVT is organized based on the drug test subversion strategies, describing how laboratories detect them. In some cases, specimen results are reported as invalid, meaning a positive, negative, adulterated, or substituted result cannot be established. These results could be caused by a subversion attempt or by a set of unusual circumstances. For example, an abnormal pH could be the result of specimen storage conditions rather than adulteration. Because there are also biological and medical reasons for invalid results, they should never be interpreted as proof of subversion. In other cases, multiple subversion strategies can cause the same SVT result. For example, "adulterated" oxidant levels could be caused by adulteration of the donor's specimen or by use of a substitution product. Because both actions represent drug test subversion, there is typically no need to determine the exact strategy used.

The Collection Process

Collectors and collection sites are the first line of defense in mitigating adulteration and substitution of specimens. Urine collection procedures minimize subversion during collection. Some examples of procedures include the following: (1) a bluing agent is used in all toilets at collection sites, (2) sources of water in the faucets are either secured or turned off, (3) donors are required to remove unnecessary clothing that might conceal adulterating substances, (4) donors are required to empty their pockets prior to collection, (5) the temperature of the urine specimen is taken upon collection, and (6) the collector visually inspects the specimen for color, foreign objects, and any other signs of adulteration such as excessive foaming.[3] All oral fluid collections are observed, but urine collections are only observed in specific instances, such as when the Medical Review Officer (MRO) reports that there is no legitimate medical explanation for an invalid result. Similar to the process for urine collections, oral fluid collections require the donor to remove unnecessary clothing and empty their pockets. Donors are also required to submit to an inspection of their oral cavity, and collectors must visually inspect the collected specimen for foreign objects, abnormal color, and other signs of adulteration.[4]

Adulteration

Urine adulterants added to a specimen either interfere with the testing process or chemically degrade the drugs or metabolites. Adulterating substances may be household cleaners, such as vinegar, bleach, or soap, or they may be items purchased for the sole purpose of adulteration, such as glutaraldehyde or nitrite.[5]

pH Adulteration

Physiologically normal urine pH ranges from 4.5 to 9.0. A specimen is considered adulterated if the pH is <4 or ≥11 and is considered invalid if the pH ranges from 4 to 4.5 or 9 to 11. A high pH can degrade some analytes. At a pH of >9.5, analytes such as benzoylecgonine, phencyclidine (PCP), and 6-acetylmorphine are not stable with losses ranging from 61% to 100% after three days at room temperature.[6] In addition, initial and confirmatory methods are typically not validated for extreme pH and may not work correctly on adulterated specimens. For example, enzymes used for hydrolysis may be less effective.

Most commonly, autoanalyzers measure pH colorimetrically, wherein a pH-sensitive dye is added to the sample aliquot, which is then measured for absorbance. Colorimetric pH testing is often performed simultaneously with the initial immunoassay drug testing. Per the UrMG, a colorimetric test may be used for initial testing only if it encompasses a dynamic range from 3.0 to 12.0. Otherwise, the colorimetric pH test is a screening test, and any abnormal results must be followed by conducting an initial pH meter analysis. A pH meter probe typically contains both an electrode and a reference electrode forming an electrochemical cell. The electric potential (voltage) of the electrode depends upon the concentration of hydronium (H_3O^+) in the solution and is measured electronically. A pH meter can be used for both initial and confirmatory pH testing and is calibrated each day by using calibrators with known pH.

Besides adulteration, pH can be affected by disease states or microbial growth. The time between collection and analysis, and the temperature of the specimen during that time, can affect microbe growth and therefore pH. As such, proper storage conditions and timely analyses of specimens are important aspects of workplace drug testing. Specimens with a pH ranging from 9 to 9.5 are more common during the summer months because the specimens are exposed to high ambient temperatures during shipment from the collection site to the laboratory.

Oxidizing Adulterants

An oxidizing adulterant act alone or in combination with other substances to interfere with initial testing through the destruction of enzymes or antibodies in immunoassay reagents, or with confirmation testing via the depletion of derivatizing agents. Oxidizing adulterants can also oxidize drugs or metabolites to change their structure and prevent detection. Examples of oxidants include nitrites, chromium (VI) compounds, halogens, or gluteraldehyde.

The UrMG require regulated specimens to undergo testing for at least one oxidizing agent. The most common assay is the general oxidant test, which is performed on an autoanalyzer and non-specifically identifies the presence of an oxidizer. The general oxidant test is semiquantitative and usually calibrated with nitrite, with results expressed in µg/mL nitrite equivalents. Nitrite can also be present in urine as a metabolite of dietary nitrates or an oxidation product of nitric oxide, but concentrations are normally

low. As such, the general oxidant assay cutoff is set at a level well above ambient levels but below those encountered with specimen tampering.

In addition to nitrite, a number of other oxidizing adulterants have been used to subvert drug testing. Chromium (VI) compounds such as chromates, dichromates, and chlorochromates are powerful oxidizing agents that have been found in products marketed to those attempting to beat a drug test. Halogens such as hypochlorite, which is found in household bleach, and iodine are strong adulterants. Glutaraldehyde is available as an over-the-counter wart treatment and is commonly used in hospital settings for sterilization. When used to adulterate a urine specimen, glutaraldehyde can interfere with immunoassays, resulting in false negative test results.

To report a specimen as adulterated, a different confirmatory test must be used (e.g., multi-wavelength spectroscopy or ion chromatography) that is specific and quantitative. The criteria used for reporting specimens as adulterated because of oxidants include the following:

- Nitrite is present at a concentration equal to or greater than 500 µg/mL in both the initial and confirmatory tests.
- Chromium (VI) is present at a concentration equal to or greater than 50 µg/mL in the initial test and at a concentration equal to or greater than the limit of quantification (LOQ) in the confirmatory test.
- Halogen is present at a concentration equal to or greater than the LOQ in the confirmatory test after indication of presence in an initial oxidant colorimetric test.
- Glutaraldehyde is present at a concentration equal to or greater than the LOQ of a specific confirmatory test after indication of presence in an initial test.

Few laboratories maintain these adulterant confirmation methods and instead repeat the general oxidant colorimetric test using a fresh aliquot or a portion of urine taken from the original urine bottle. Obtaining consistent results from the colorimetric test on a second aliquot enables the specimen to be reported as invalid.

Surfactants

A surfactant lowers the surface tension of a liquid, increasing contact between the liquid and another material, effectively creating an emulsion of dirt and oils and preventing the particles from settling back into the solution. Surfactants, which are found in cosmetics and in cleaning and oil recovery products, can be used to subvert drug testing by altering pH and ionic strength, increasing drug-binding to antibodies or by forming an insoluble complex with drug and/or metabolite. The UrMG do not require routine testing for surfactants, but some surfactant can be detected using a methylene blue colorimetric procedure. A laboratory may report a specimens as adulterated because of the presence of a surfactant when a dodecylbenzene sulfonate–equivalent concentration greater than or equal to 100 µg/mL is detected in a colorimetric test and a specific confirmatory test using a different method. Many laboratories do not maintain testing methods for surfactants; however, laboratory staff should note abnormal physical characteristics such as excessive bubbling or foaming that may indicate adulteration with surfactants.

Interference on Initial or Confirmatory Drug Tests

Many laboratories do not maintain the technology to detect and quantitate specific adulterants; however, they may still be able to detect the effects of them through general interference with immunoassay initial tests. Adulterants change the chemical makeup of the urine specimen, which sometimes interferes with the chemistry of the immunoassay to generate a response even lower than a negative urine specimen. This phenomenon is called immunoassay suppression and is sometimes referred to as "super-negative" specimens. Laboratories must have procedures in place to identify immunoassay interference when it occurs.

A laboratory is allowed to report a specimen as invalid if interference occurs on two separate aliquots in either the initial test or the confirmatory test. In the case of the initial test, the interference is usually detected as "super-negative" results in one or more assays, where the instrument reading is substantially different from the blank. Such readings can be caused by the presence of an adulterant interfering with the antibody or color reaction of the assays. In the case of the confirmatory test, the interference is usually detected as a chromatographic peak that interferes with the analyte peak so that identification criteria, such as peak shape, resolution, or ion ratio, cannot be met.

Substitution

Subversion of a urine drug test may be achieved through substitution of the complete specimen with a negative specimen by using either authentic urine from a different individual or synthetic urine. The market for synthetic urine is large, and products with professional-looking packaging can easily be obtained (**Exhibit 1**). Many synthetic urines are able to pass SVT,[7] and presumably many authentic specimens from a different individual would too. Substitution of a urine specimen with another material entails bringing the urine substitute into the restroom at a collection site without detection and ensuring that the specimen can pass a visual and temperature inspection. When obtaining specimens, it is paramount that the collector uses proper collection procedures and consistently demonstrates vigilance because that is the most effective way to prevent substitution.

Exhibit 1. *Packaging and contents of select synthetic urine products, purchased in December 2024*

Creatinine and Specific Gravity

After a specimen has been collected, creatinine and specific gravity tests are used to determine whether a specimen is substituted, dilute, or invalid. Specific gravity is only measured if creatinine is <20 mg/dL and depending on the combination of results, the specimen can be reported as substituted, invalid, dilute, or within normal range (**Exhibit 2**).

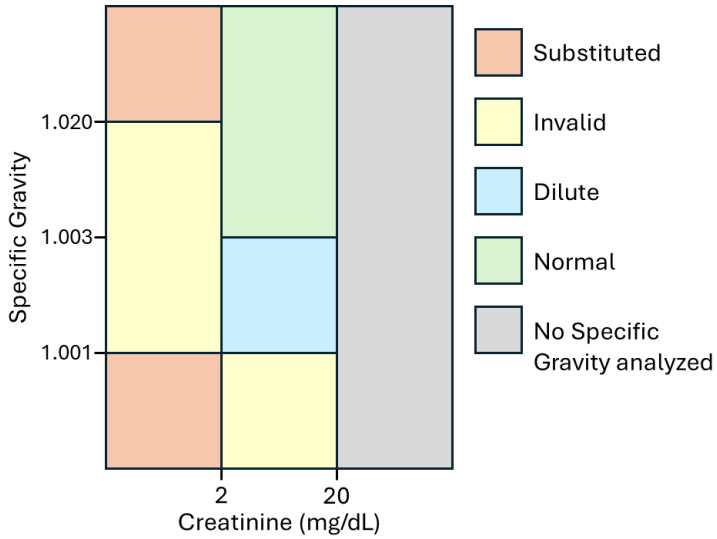


Exhibit 2. *Relationship between creatinine and specific gravity*

Creatinine in urine primarily originates from muscle metabolism but it may also come from dietary protein. In muscle cells, creatine is converted to phosphocreatine to facilitate muscle contraction; both creatine and phosphocreatine are metabolized to creatinine. The elimination of creatinine in urine remains relatively constant day to day; therefore, it is a common clinical measure of renal function. Normal urine creatinine ranges from 20 to 275 mg/dL in women and 20 to 320 mg/dL in men.

The Jaffe reaction is often performed with an autoanalyzer and is used as both an initial and a confirmatory creatinine test; a second aliquot is required for confirmation testing. The Jaffe reaction is a kinetic reaction between creatinine and picric acid. In the presence of a base, creatinine and picric acid will form a chromogen that absorbs light ranging from 480 to 520 nm.

Specific gravity is a measure of the concentration of waste products, such as salts, glucose, urea, and proteins, excreted in urine. The specific gravity of urine is defined as the density of the sample divided by the density of water at a given temperature. A more concentrated urine specimen will have a higher specific gravity, whereas a less concentrated urine specimen will have a lower specific gravity closer to 1.0000.

The UrMG separate specific gravity measurements into the following two classes based on the refractometers used: three-decimal place and four-decimal place. Three decimal–place refractometers are often small handheld or benchtop instruments, and guidelines stipulate their use for screening only. Four decimal–place refractometers may be used for both initial and confirmatory tests, but a separate aliquot must be used for the confirmatory test.

Liquid Intake and Detoxing

A tried and trusted method that donors use to improve the chances of a negative result is to drink large amounts of water before being tested. Consuming large amounts of water will increase urine output and consequently lower the concentrations of drugs and metabolites in the specimen. If creatinine and specific gravity are lower than expected, then the specimen will be reported as dilute, which indicates that the specimen may not provide an effective evaluation of recent drug use. A dilute specimen is not considered evidence of drug use, but this result will typically trigger a recollection. Sometimes donors consume vitamin B12 (hydroxocobalamin) to color the urine a bright yellow color, or ingest creatine monohydrate in an attempt to increase creatinine measurements.[2] There is no evidence that either strategy is effective in altering or subverting the SVT.

Another strategy that donors use before testing is to utilize ingestible aids purchased online or at vitamin shops, with the goal of increasing drug elimination or reducing drug levels in the specimen, mainly focusing on cannabis metabolites during the hours and days leading up to the test. Abstinence, which is a key part of these strategies, is effective in decreasing metabolite concentrations in urine, but there is no evidence to suggest that the ingestible aids have any effect.

Oral Fluid Subversion

Currently, there is no required SVT for oral fluid specimens because all oral fluid collections are observed,[8] thus making it difficult for donors to substitute or adulterate the specimen. Instead, oral fluid testing subversion focuses on oral hygiene, in which donors vigorously brush their teeth and gums and use mouthwash and specialized subversion products to remove drugs prior to the test.[2] Although the efficacy of subversion products is unclear, oral hygiene is likely to be helpful. For example, Δ9-tetrahydrocannabinol (THC) in oral fluid may result from oral cavity contamination as opposed to an equilibrium with circulating drug, yielding high THC concentrations shortly after cannabis use.[9]

Conclusion

The UrMG require SVT in order to determine the authenticity of urine specimens tested under the Drug-Free Workplace Testing Program. These tests help identify and deter adulteration, substitution, and detoxing and aid in public safety by ensuring that our safety-sensitive federal employees remain drug-free. The use of synthetic urine is a large contributor to drug test subversion and is rarely detected by SVT; as such, collectors and personnel at collection sites must take appropriate actions to protect the integrity of specimens on the front line. As a second line of defense, ongoing research seeks to identify other indicators of specimen authenticity, including biomarker testing.

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