PharmacologyOnLine April 30, 2014

Archives • 2014 • vol.1 •102-108

False Positive and False Negative Results in Urine Drug Screening Tests: Tampering Methods and Specimen Integrity Tests

Bamdad Riahi-Zanjani

Medical Toxicology Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

riahib@mums.ac.ir; bamdad_riahi@yahoo.com

Abstract

Urine drug screening can detect cases of drug abuse, promote workplace safety, and monitor drug-therapy compliance. Compliance testing is necessary for patients taking controlled drugs. To order and interpret these tests, it is required to know of testing modalities, kinetic of drugs, and different causes of false-positive and false-negative results. Standard immunoassay testing is fast, cheap, and the preferred primarily test for urine drug screening. This method reliably detects commonly drugs of abuse such as opiates, opioids, amphetamine/methamphetamine, cocaine, cannabinoids, phencyclidine, barbiturates, and benzodiazepines. Although immunoassays are sensitive and specific to the presence of drugs/drug metabolites, false negative and positive results may be created in some cases. Unexpected positive test results should be checked with a confirmatory method such as gas chromatography/mass spectrometry. Careful attention to urine collection methods and performing the specimen integrity tests can identify some attempts by patients to produce false-negative test results.

Keywords: Urine drug screening, False negative, False positive, Specimen integrity tests

Introduction

Drug abuse is a critical problem throughout the world. Urine drug screening is commonly required as a workplace mandate. It may also be required for marriage, military or sports participation; for legal or criminal investigations; or drug-therapy compliance monitoring. For this reason, abusers always try to hide their abuse (1, 2). Urine drug test, in several countries is the most primarily available qualitative test for identifying drug abusers. Since the users of illicit drugs try to defeat and manipulate urine specimens to pass drug testing, applying some strategies can lead to detection of their fraud. Widely abused drugs are opiates, amphetamine, cocaine, cannabinoids, phencyclidine, and benzodiazepines. Some drugs such as Ecstasy, flunitrazepam, and γ-hydroxybutyric acid are also widely used in rave parties (2).

Urine drug screening tests (UDSTs) are generally used to detect common drugs of abuse in urine samples. Although blood, hair, nails, or saliva can be used, most screening is performed on urine samples. Convenience of collection, higher volumes, higher drug concentrations, and longer (sufficient) durations of detection are primary reasons for using of urine sample in drug detection (3). Since some of personal, occupational, and legal implications accompany drug testing, family technologists who perform UDSTs must be able to interpret screening results and respond appropriately to that interpretation. To order and interpret UDSTs, it is required to know the different testing modalities, the detection times for specific drugs, kinetic of drugs and the common reasons for false-positive and false-negative test results.

Testing methods

Screening tests

UDSTs are generally performed using immunoassay. They are planned to isolate negative samples from samples which are "presumptively" positive. Immunoassay UDSTs include specific antibodies against common drugs of abuse (and their metabolites) and recognize specific structural features common of the drug. The immunoassay is the most widely used UDS because its convenience and cost efficiency. Five different immunoassays are available: enzyme linked immunosorbent assay, enzyme-multiplied immunoassay, fluorescence polarization immunoassay, immunoturbidimetic and radioimmunoassay (4). Instant on-site drug testing is another form of UDSTs that is rapid, easy to perform and give reproducible results. Many of these have been modified for increased sensitivity and can be made semi quantitative for use in a clinical laboratory by reading the color reaction with a densitometer (5). The substances most widely tested by a typical immunoassay technique include amphetamines, cannabinoid metabolites, cocaine metabolites, opiate metabolites, and phencyclidine. There are also expanded immunoassays to experiment tricyclic antidepressants, barbiturates, methadone, and benzodiazepines. They may be useful when use of these substances is suspected. However, one major problem with immunoassays is false-negative and false-positive results (1).

Confirmation test

Preliminary positive test results from an immunoassay test should be confirmed using a confirmatory test such as GC/MS or HPLC. So, a more specific confirmatory test, such as GC-MS, is required to approve a positive test result with an immunoassay. GC-MS is more accurate and precise than an immunoassay. However, it is more expensive and time consuming (1). GC-MS crashes drug molecules into ionized segments and analyzes substances based on mass-to-charge ratio using by a mass spectrometer.

General consideration over immunoassay screening test

As mentioned above, UDSTs are generally performed on urine samples using immunoassay in many of countries. Since the basis of immunoassay technique in drug detection is based on antigen and antibody reaction, one the most important reasons for the production of false negative and positive results is associated with factors that may interfere at the Ag–Ab complex formation. For example, the optimum PH for the formation of antigen-antibody complex is 6.5 up to 7. At pH 5.0 or 9.5, the equilibrium constant of Ag-Ab reaction equation is 100-fold lower than at 6.5–7.0 (6). At both sides of the maximum, the antigen-antibody reaction is strongly inhibited.

Extreme pH values induce marked conformational changes in the antibody molecule that probably destroy the complementarity with the antigen (7, 8). Therefore, if substances which are added to urine environment are able to reach PH environment higher or lower than 9.5 or 5, respectively, the reaction between Ag and Ab may not be formed and a false negative result created. Another important factor which can affect on Ag-Ab reaction is Ionic strength of urine environment. The ionic strength is the normality of environment. If it is highly changed so that an antigen or antibody conformation changes occurs, the binding of Ag and Ab may not be created. For example adding appropriate NaCl to urine environment may cause a false negative result (7, 8).

All UDSTs used in labs have cut off levels. It is a level of substance in the system that is considered as a positive if the substance is revealed at this concentration. The United States Department of Health and Human Services (DHHS) sets the threshold for drug concentrations for detection by UDS (3). Drug concentrations in the urine below this level are reported as negative. Table 1 contains a list of these values with their detection times.

False positive results

Although immunoassay techniques are so sensitive to the presence of drugs/drug metabolites, specificity differs depending on the assay used and the drug for detection (3). Specificity, the most important characteristic of the antigen-antibody reaction, is determined by the antigen identified by the complementary nature of the spatial structure between the cluster and the hyper variable area of the antibody molecules. Consequently, patients who use agents that are similar to drug target in terms of spatial structure may receive a false-positive test result.

This limitation may result in false-positives from substances cross-reacting with the immunoassay (9-11). For example, selgiline is metabolized to *l*-amphetamine and *l*-methamphetamine, isomers without central nervous system stimulation. Therefore, if a urine sample of a patient using selgiline is tested for amphetamines, it (selgiline metabolite) may cause a false positive for amphetamines (9).

In contrast, the test results of abusers who use agents that are not similar to drug target (drug under detection of immunoassay kit) in conformational structure may become negative. They may abuse other substances. Positive results observed on immunoassay required to be approved using the GC-MS which is more accurate than immunoassay (1). The DHHS detection limits have reduced false-positive results, but have not eliminated them. In 1998, the cut-off level for opiates was elevated from 300 ng/mL to 2000 ng/mL because of avoiding false positives from poppy seed ingestion. However, these more strict limits may lead to false-negative results and a lot of laboratories go on to use the lower value for detection. For example, detectable levels of cannabinoids after consumption of hemp-containing foods with immunoassay have been reported. While, concentrations of cannabinoids in these samples were not detected with GC-MS. Passive marijuana or cocaine smoke inhalation has not been reported to achieve detectable urine concentrations in adults. However, passive cocaine smoke inhalation has caused detectable levels in pediatric cases (9-11).

Many materials have been reported to cross-react with immunoassays and cause false-positives. Most have only been documented in case reports. Table 2 shows substances reported to cause false-positive results using immunoassay. This list may not include all potential substances. The frequency of false-positive results depends on the specificity of immunoassay used and the drug under detection. Immunoassay results for marijuana and cocaine metabolites are related to very few false-positives while the results for amphetamines and opiates are related to a higher number of false-positives (1). Finally, to reduce false positive results, data obtained from an immunoassay test should be checked by gas chromatography/mass spectrometry or high-performance liquid chromatography (1).

False negative results (Urine manipulation & Specimen integrity tests)

False negatives can happen as a result of low levels of urine drug, manipulation, and in other situations. Time since drug usage, amount and frequency of use, fluid intake, body fat level, and metabolic factors can affect on the urine drug concentration (1).

There are many ways for patients to tamper testing. These include adding adulterants to urine at the time of testing, urine dilution through drinking excessive water, consumption of substances that interfere with testing, and substitution of a clean urine sample (1, 3, 12, 13).

A lot of drug abusers try to tamper drug tests by adding readily household products such as tablet salt, vinegar, bleach, and drain cleaner to urine samples to pass drug tests. Since the high scale variation of environment pH and ionic strength are destructive on the reaction between antigens and antibodies, many household substances cause false-negative results with this mechanism. Table 3 lists some cases that may cause false-negative results in urine drug immunoassay screening test. Different strategies can be applied to realize the presence of these adulterants in urine samples. Some cases are traceable because of variations they cause in the appearance, specific gravity, or pH of the urine. Adulteration strips or simple tests can detect the levels of acidity and ionic strength in the urine (12-23).

The easiest way for drug users to pass drug tests is substituting urine samples. Clean urine of abuse drugs is covertly poured into the urine collecting cup to pass drug tests (12). An artificial penis with an electronic, temperature-controlled urine reservoir can be purchased online. Patients may try to elude detection by voiding before testing, then refilling their bladder with clean urine using a catheter (23).

Exact viewing over the collecting site during voiding can prevent this method and disappoint abusers from substituting urine samples. Some of drug testing devices have temperature strips located in their collecting cups, which can show coolness of the sample.

Directly diluting urine samples or drinking massive amounts of water (or use of diuretic drugs) is the other easy methods that drug users do to cheat urine drug tests. This method, called dilution, is used to dilute urine with normal water with the hopes of lowering the level of drugs so that it passes the cut-off levels of the drug test (12, 13). This can usually be detected if the urine sample is very clear in appearance or if the temperature of the urine falls below normal. Creatinine levels and specific gravity are the best tests to be checked to detect the possibility of urine dilution. Therefore, excessively dilute samples should be rejected.

There are commercially available detoxifying kits that claim to clean urine and help to alter test results. *"Stealth" (peroxide/peroxidase)*, *"Urine Luck" (Chromate, pyridinium chromate)* and *"Urine clear" (Sodium nitrite)* are examples of detoxifying agents that work with different mechanisms (24-27). These agents can oxidize and change the structure of abuse drugs so that antibodies used in kits are not be able to detect drugs. Routine specimen integrity testing involving pH, creatinine, specific gravity, and temperature is inadequate for detecting the presence of more recently introduced adulterants such as Urine Luck, Clear, and Stealth. However, the detection of these adulterants is possible by Dip Sticks and wet chemistry tests (1, 24-27). Some of laboratory findings that suggest adulteration, dilution and substitution of the urine samples are shown in table 4. Some of strategies which can be applied to detect urine manipulation are seen in table 5.

Finally, some of false negatives are created from inappropriate tests used. Understanding the UDS and ordering the appropriate test can prevent false-negative results. Results from an immunoassay can be deceiving, as these tests may not be able to detect every drug in a particular drug class.[2] For example, a test for opiates will detect morphine and drugs that are metabolized to morphine, such as codeine and heroin. Other opiates (opioids) such as fentanyl, oxycodone, methadone, hydrocodone, buprenorphine, and tramadol will not be detected and require an expanded immunoassay panel for detection (1).

Conclusion

In conclusion, to reduce false-positive and false-negative test results, (10-13), it is firstly recommended to perform appropriate collection techniques and tests of specimen integrity can reduce the risk of tampering/false negatives (30-32) and secondly select a test panel based on the compounds seeking to detect. An expanded drugs of abuse panel is needed to detect other commonly used drugs, including opiates, opioids, amphetamine/methamphetamine, cocaine, cannabinoids, benzodiazepines and etc (33). Finally, to reduce false positive results, data obtained from an immunoassay test should be checked by gas chromatography/mass spectrometry or high-performance liquid chromatography.

References

1. Standridge JB, Adams SM, Zotos AP. Urine drug screen: a valuable office procedure. *Am Fam Physician* 2010;8:635-640.
2. Dasgupta A. The Effects of Adulterants and Selected Ingested Compounds on Drugs-of-Abuse Testing in Urine. Am J Clin Pathol 2007;128:491-503.
3. Moeller KE, Lee KC, Kissack JC. Urine drug screening: practical guide for clinicians. *Mayo Clin Proc.* 2008;83(1):66-76.
4. Liu RH Comparison of common immunoassay kits for effective application in workplace drug urinalysis. Foresic Sci Rev 1999;6:19-57.
5. Levy S, Sherritt L, Vaughan BL, Germak M, Knight JR. Results of random drug testing in an adolescent substance abuse program. Pediatrics 2007;119:843-8.
6. Barnes AE. The specificity of pH and ionic strength effects in the kinetics of the Rh(D)-anti-Rh(D) system. J Immunol 1966;96:854–64.
7. Hughes-Jones NC, Gardner B, Telford R. Comparison of various methods of dissociation of anti-D, using ^{131}I-labelled antibody. Vox Sang 1963;8:531–6.
8. Howard PL. Principles of antibody elution. Transfusion 1981;21:477–82.
9. Vincent EC, Zebelman A, Goodwin C. What common substances can cause false positives on urine drug screens for drugs of abuse? *J Fam Pract* 2006;55:893-894.
10. Brahm NC, Yeager LL, Fox MD, Farmer KC, Palmer TA. Commonly prescribed medications and potential false-positive urine drug screens. *Am J Health-Syst Pharm* 2010;67:1344-50.
11. Woelfel JA. Drug abuse urine tests: false-positive results. *Pharmacist's Letter/Prescriber's Letter.* 2005;21:210314.
12. Lafolie P, Beck O, Blennow G, Boreus L, Borg S, Elwin CE, Karlsson L, Odelius G, Hjemdahl P. Importance of creatinine analyses of urine when screening for abused drugs. Clin Chem 1991;37:1927-31.
13. Cone EJ, Lange R, Darwin WD. In vivo adulteration, excess fluid ingestion causes false-negative marijuana and cocain urine test results. J. Anal Toxicol 1998; 22:460.
14. Garside D, Callies JA. Analysis of Urine for Anionic Surfactants. J Anal Toxicol 2001; 25:368.
15. Baiker C, Serrano L, Lindner B. Hypochlorite Adulteration of Urine Caising Decreased Concentration of D9-THC-COOH by GC/MS. J Anal Toxicol 1994;18:101-3.
16. Critchfield GC, Wilkins DG, Loughmiller DL, Davis BW, Rollins DE. Antibody-mediated interference of a homogeneous immunoassay. J Anal Toxicol 1993;17: 69-72.
17. Cody JT. Specimen adulteration in drug urinalysis. Forensic Sci Rev 1990;2:63-75.
18. Cody JT. Adulteration of urine specimens, Handbook of Workplace Drug Testing, R.H. Liu and B.A., Eds. AACC Press, Washington, D.C., 1995;181-208.
19. Davis KH. Adulterants update, Society of Forensic Toxicologists annual meeting, San Juan, Puerto Rico, 1999.
20. Pearson S, Ash K, Urry F. Mechanism of false negative urine cannabinoid immunoassay screens by Visine eyedrops. Clin Chem 1989;35:636-8.
21. Warner A. Interference of common household chemicals in immunoassay methods for drugs of abuse, Clin Chem 1989;35:548 – 51.
22. George S, Braithwaite R. An Investigation into the extent of possible dilution of specimen received for urinary drugs of abuse screening, addiction. 1995;9:967-70.
23. Jaffee WB, Trucco E, Levy S, Weiss RD. Is this urine really negative? A systematic review of tampering methods in urine drug screening and testing. J Subst Abuse Treat 2007;33:33-42.
24. Colbourne PD, Boisvert YM, Parent S, LeGatt D, KiniburghDW. Chromate adulteration in Employment-Related Drug Screens. J Anal Toxicol 2001;25:369.
25. Tsai JS, ElSohly MA, Tsai SF, Salamone SJ. Modulation of Oxidizing Agents Adulteration by Manipulation of Urinary pH Values. J Anal Toxicol 2001;25:368.
26. Valtier S, Cody JT. Characterization of the Effects of Stealth Adulterant on Drugs-of-Abuse Testing. J Anal Toxicol 2001;25:369.
27. Cody JT, Valtier S. Effects of Stealth Adulterant on Immunoassay testing for Drugs-of-Abuse. J Anal Toxicol 2001;25:466-70.
28. AGSA, Swiss Working Group for Drugs of Abuse Testing Guidelines, www.consilia-sa.ch/agsa/
29. SAMHSA Guidelines, Substance Abuse and Mental Health Services Administration (SAMHSA), www.samhsa.gov/library/searchreal.aspx
30. Jaffee WB, Trucco E, Levy S, Weiss RD. Is this urine really negative? A systematic review of tampering methods in urine drug screening and testing. J Subst Abuse Treat. 2007;33:33-42.
31. U.S. Department of Health and Human Services. Substance Abuse and Mental Health Services Administration
32. Division of Workplace Programs. Urine specimen collection handbook for federal agency workplace drug testing programs. November 2004. http://workplace.samhsa.gov/DrugTesting/Level_1_Pages/HHS%20Urine%20Specimen%20Collection%20Handbook%20(Effective%20November%201,%202004).aspx. Accessed January 7, 2009.
33. Specimen Validity Testing. Substance Abuse and Mental Health Services Administration Division of Workplace
34. Programs. February 2005. http://workplace.samhsa.gov/DrugTesting/Files_Drug_Testing/Labs/Specimen%20Validity%20Testing%20-%20February%202005. pdf. Accessed January 7, 2009.
35. Moeller KE, Lee KC, Kissack JC. Urine drug screening: practical guide for clinicians. Mayo Clin Proc 2008;83:66-76.

Table 1. Cut off levels along with detection times for urine immunoassay screening tests.

Drug category	Cut off level (ng/ml)	Detection time
Amphetamine/Methamphetamine	1000	2 days
Benzodiazepines	300	3-30 days
Cocaine metabolite	300	2-4 days
Opiates	2000	2-4 days
Barbiturates	300	21 days
Phencyclidine	25	8 days
Marijuana (Δ_9-THC-COOH)	50	3-30 days

Table 2. Agents that may create false-positive results in urine drug immunoassay screening test.

Drug category	Interfering drugs
Amphetamines	Amantadine, bupropion, chlorpromazine, ranitidine, desipramine, fluoxetine, labetalol, methylphenidate, phentermine, phenylephrine, phenylpropanolamine, promethazine,pseudoephedrine, trazodone
Benzodiazepines	Oxaprozin, sertraline
Cocaine	Topical anesthetics containing cocaine
Opiates	Dextromethorphan, diphenhydramine, quinine, Fluoroquinolones, poppy seeds, rifampin,
Barbiturates	Ibuprofen, naproxen
Phencyclidine	Dextromethorphan, diphenhydramine, ibuprofen, imipramine, ketamine, meperidine, thioridazine, tramadol, venlafaxine
Cannabinoides	Dronabinol , nonsteroidal anti inflammatory drugs, proton pump inhibitors

Table 3. Cases that may cause false-negative results in urine drug immunoassay screening test.

Infrequent drug use
Prolonged time since last use
Recent use
Insufficient amount ingested
Inappropriate test used
Manipulation
• Dilute urine (excess fluid intake, diuretic usage, adding water to urine sample)
• Substitution with a normal urine
• Bleach
• Vinegar
• Soap
• Ammonia
• Lemon juice
• Drain cleaner
• Sodium chloride
• Tetra hydrozoline (eye drops)
• Various chemicals (glutaraldehyde, nitrite, pyridinium chlorochromate, and peroxide/peroxidase)

Table 4. Laboratory findings that suggest urine manipulation (28, 29).

Cases suggestive of adulteration, dilution, or substitution of the samples
General
• Temperature < 90° F or >100° F
• Abnormal appearance (cloudy, dark)
Adulteration
• Nitrite > 500 mg/dl
• pH < 3 or pH > 11
Dilution
• 20 mg/dl < Urine creatinine ≥ 2 mg/dl
• 1.001< Specific gravity < 1.003
Substitution
• Urine creatinine < 2 mg/dl
• Specific gravity ≤ 1.001 or ≥ 1.030

Table 5. Methods/substances of manipulations that interferences in Immunoassays screening tests along with their integrity tests (12-27).
[a]ACR = Adulteration screening reagents (wet chemistry)

Method/substance	Integrity test
Urine exchange/External urine	Should be controlled during void
Taking of diuretic substances	Creatinine, specific gravity
Sodium chloride	Analysis of sodium or chloride
Bleaching agent	pH, smell, ACR[a]
Detergents	Foaming test, pH, ACR
Vinegar, drain cleaner, baking soda and ammonia	pH
Dilution of urine by excessive fluid intake	Creatinine, specific gravity
Chromate, Pyridiniumchromates	Atomic absorption for chromate, ACR
Peroxide/Peroxidase	pH, ACR
Nitrite	ACR