

Common Interferences in Drug Testing



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KEYWORDS

- False positive • False negative • Drugs • Toxicology • Forensic • Workplace
- Laboratory

KEY POINTS

- Interferences relating to laboratory toxicology testing refer to results which differ from their true value and are often encountered in the setting of a drug screen compared with confirmatory testing results.
- Interferences fall into two general categories; those that cause false positive results (when a drug screen is positive but confirmatory testing is negative) and those that cause false negative results (when a drug screen is negative when in reality the sample donor has ingested the tested substance).
- Interferences can result from differences in laboratory testing methodology, reagent and analyte cross reactivity, limits of analyte detection, instrument resolution, reporting cutoff, sample processing, tissue type and sample adulteration among others.
- Awareness of the possible causes of such interferences are integral to proper laboratory result interpretation and patient management.

Interferences with toxicology testing fall into two categories: those that cause false-positive results and those that cause false-negative results. The terms *false positive* and *false negative* in the context that follows refers to the screening test result as compared with the true result; that is, a false-positive result is a result that is screen positive for a particular class of drugs, when in reality, the donor has not ingested any of those substances. Conversely, a false-negative result is when a sample screens negative for a class of drugs, when in reality, the donor has ingested one of the tested substances.¹

False-positive screen results are not a major concern for most toxicology laboratories, as confirmatory testing will resolve the screening discrepancy. Confirmation

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testing is always more sensitive and specific than the initial screening test. False-negative results are a significant concern, however, as a sample that screens negative will not be sent for confirmatory testing. This issue becomes a concern because if the donor has intentionally masked the ingestion of a drug, the testing will not reveal it.

It should be noted that interferences only occur with the initial testing, which is usually immunoassay. Mass spectrometry confirmation tests are not affected by interfering substances or cross-reacting drugs.²

FALSE POSITIVE

False-positive interferences are usually drugs or other substances that are often structurally related to the class of drugs that is being screened for. Antibodies in the screening reagent are designed to detect common epitopes, particularly in drug classes with many substances (opioids, benzodiazepines, sympathomimetic amines). This design allows the test to be able to detect the many different drugs within a particular class. However, sometimes substances may be structurally similar to the intended class and, thus, are inadvertently detected by the assay. There is most often no intent on the part of the sample donor to cause a false-positive result.^{3,4} **Table 1** describes commonly known drug interferences.^{4,5}

FALSE NEGATIVE

False-negative interferences may or may not be intentionally ingested to attempt to mask the ingestion of a drug patients do not want to be detected. False-negative tests are more of a concern because, based on most toxicology laboratories' testing scenarios, negative screening samples are not investigated further.

Dilution

The simplest of these interferences involves diluting one's urine to the point that the concentration of the drug is less than the detection limits of the test. Patients either add additional liquid to the urine sample or drink something that causes their urine to be very dilute. Although this is a very effective technique, it is easily detected by the testing facility when they test the creatinine and specific gravity or observe the collection process. A normal urine has a creatinine greater than 20 mg/dL, along with a specific gravity that is greater than 1.030. If a urine specimen has both creatinine and specific gravity values less than these cutoffs, the urine is considered to be dilute.⁶

Substitution

Patients can attempt to substitute their urine with either someone else's urine or with a urinelike liquid. This type of interferences is detected with an observed collection, recording the temperature of the urine immediately after collecting, and/or the testing facility testing the creatinine and specific gravity of the sample. A sample that is not between 90°F and 100°F is not humanly possible and should be questioned as to the source of the specimen (Nuclear Regulatory Commission, Regulations Title 10, Code of Federal Regulations, part § 26.111). Additionally, a creatinine that is less than 2 mg/dL with a specific gravity that is less than or equal to 1.0010 or greater than or equal to 1.030 is not physiologically possible and is, thus, considered to be substituted.⁶

Adulterated

Another way a sample can be tampered with is by the addition of a substance that interferes with screening test that is causing the antibody to not bind to the drug. Specimen validity testing for oxidative substances and the pH of the sample usually detects

Table 1
Drugs that cause false-positive immunoassay screens

Drug Screen	Interfering Substance
Amphetamines	Amantadine Benzphetamine Bupropion Chlorpromazine Clobenzorex l-deprenyl Desipramine Dextroamphetamine Ephedrine Fenproporex Isometheptene Labetalol MDMA Methamphetamine L-Methamphetamine (Vick's inhaler) Methylphenidate Phentermine Phenylephrine Phenylpropanolamine Promethazine Pseudoephedrine Ranitidine Ritodrine Selegiline Thioridazine Trazodone Trimethobenzamide Trimipramine
Benzodiazepines	Oxaprozin Sertraline
Cannabinoids	Dronabinol Efavirenz Hemp-containing food NSAIDs Proton pump inhibitors Tolmetin
Cocaine	Coca leaf tea Topical anesthetics containing cocaine
Opioids	Dextromethorphan Heroin Poppy seeds Quinine Quinolones Rifampin Verapamil

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Table 1 (continued)	
Drug Screen	Interfering Substance
PCP	Dextromethorphan Diphenhydramine Doxylamine Ibuprofen Ketamine Meperidine Mesoridazine Thioridazine Tramadol Venlafaxine

Abbreviations: MDMA, 3,4-methylenedioxymethamphetamine; NSAIDs, nonsteroidal antiinflammatory drugs; PCP, phencyclidine.

Data from Johnson-Davis KL, Sadler AJ, Gnzen JR. A retrospective analysis of urine drugs of abuse immunoassay true positive rates at a national reference laboratory. *J Anal Toxicol* 2016;40:97–107; and Brahm NC, Yeager LL, Fox MD, et al. Commonly prescribed medications and potential false-positive urine drug screens. *Am J Health Syst Pharm* 2010;67:1344–50.

these and other types of interferences.⁷ A specimen will be reported as substituted when one of the following criteria is met:

- pH less than 3 or 11 or greater
- Nitrite \geq 500 mcg/mL
- Chromium (VI) is present
- A halogen (eg, bleach, iodine, fluoride) is present
- Glutaraldehyde is present
- Pyridine is present
- A surfactant is present

Collection Process

The observed collection is the process of searching patients before the collection process as well as observing the patients providing the urine specimen. For obvious reasons, this process can cause both the patients and the collector to become uncomfortable. It is the collector's responsibility to strictly adhere to the observed collection protocols to ensure the sample is valid. This protocol includes asking potentially difficult or embarrassing questions as well as ensuring items, such as prosthetic limbs, do not contain substituted urine or other adulterating substances.⁸ There is variability among clinical collection sites with regard to adherence to ascertaining whether strict collection protocols are part of a standard operating procedure and enforced. Thus, careful assessment of collection processes in light of an inconsistent laboratory toxicology result can often shed light as to whether adulteration or specimen mishandling, including accidental patient specimen mix-up, may have taken place.

Sample Processing

Once the sample reaches the laboratory, noting any odors or unusual appearance can be clues that the sample has been tampered with. Excessive foam, lack of color or a greenish-blue color, or the odors of bleach are all indications that the sample has been tampered with.⁸

Besides intentionally creating a false-negative response, many questions can arise from the unintentional false-positive result. This result most often occurs because of

cross reactivity between substances within the same class or a structurally similar drug. This cross reactivity causes a difficult situation for health care providers or law enforcement/probation officers, particularly when the person being tested has a history of drug abuse. There are also issues that arise when samples are stored for extended amounts of time as well as when multiple different sample types are submitted for analysis on the same individual. Adherence to a strict standard operating procedure with respect to collection, affirmation of patient/sample pairing, packaging and processing as well as accounting for human fatigue can help obviate such types of preanalytical sample procurement errors.

There is nothing that can replace the information that is obtained by taking a thorough history and background. Additionally, the laboratory cannot be tasked with carrying the entire burden of the drug testing result. It must be clearly understood that the clinical toxicology laboratory provides objective ancillary support, which should be used in concert with other clinical parameters toward the effective management of patients and should not be a standalone measurement in this regard. It is the responsibility of the provider to investigate when the testing result is not what was expected. Literature review, questioning patients, or even a call to the laboratory to ask for clarity in unusual situations may be warranted. In most such situations the error can be identified through careful inspection of the process. However, there are times when such assessment does not yield clarity. In such cases, retesting of the patients, either scheduled and/or unscheduled with careful attention to their drug ingestion scheduling, polypharmacy, collection of sample, and so forth can help in this regard.

Sample Type

The use of different sample types can lead to confusion when performing drug testing. The reason is simple human physiology. The different sample types represent different phases of the absorption, distribution, metabolism, and excretion (ADME) process. Depending on the time of ingestion, not all sample types can be expected to be positive. The sample types most often submitted for analysis are urine, blood, hair, and oral fluid. There are more if postmortem analysis is considered, but that is beyond the scope of this review.

If multiple different specimen types are submitted for testing on the same patient/subject, it is possible for some to misinterpret the results. For example, the most common dual specimen submission is urine and blood. The window of detection for blood can differ from that of urine for many analytes. One only has to consider the principles of ADME to understand why a urine test could be positive at the same time a blood test is negative. Blood passes through the liver at an approximate rate of 1.5 to 1.8 L/min.⁹ Thus, in the typical 70-kg patient with an anticipated blood volume of about 5 L, the total blood volume would traverse the liver every 2.8 to 3.3 minutes. Further, each drug's half-life (in addition to variations in catabolic enzyme type and efficiency) would affect the rate of catabolism. The liver is one of the principal organs that removes the drug from the blood and can further metabolize the parent drug to something that the body can easily excrete in the urine. Certain unique parent-metabolite profiles are often detectable, thus, making urine an ideal specimen for testing for recent use of substances, up to 96 hours after ingestion, for most analytes. For example, ethanol detection in urine does not correlate with a similar presence and concentration in blood. Studies by Winek and colleagues¹⁰ reported a disparity in the ethanol urine/blood ratios ranging from 0.7:1 to 21:1 demonstrating that presence and concentration in one fluid cannot necessarily predict such in another fluid. Similarly, buprenorphine analysis can be affected by disease states, such as end-stage renal failure, whereby blood/plasma concentrations of the two inactive metabolites,

norbuprenorphine and buprenorphine-3-glucuronide, were found to be increased by 4 and 15 times, respectively, in subjects with renal failure, whereas urine concentrations were not reported to be affected. This finding is likely because renal elimination plays a relatively small role (less than 30% after intravenous administration) in the overall clearance of buprenorphine.¹¹ Such an understanding would be necessary for interpretation in patients with such disease states as discussed later in this review. **Table 2** lists the characteristics of the 4 most common specimen types used in toxicology testing.^{12,13}

Instrumentation

Questioning a drug screen result is not uncommon and neither is repeating said test at a different facility. However, before comparing results from 2 different laboratories, it is imperative that the physician knows the technology each laboratory uses. A growing trend is for laboratories to forgo the immunoassay screen and perform only a Liquid

Specimen Type	Window of Detection	Benefits	Limitations
Oral fluid	0–50 h	No need for medical facility Can be done anywhere Difficult to adulterate sample	Detection limited to single-dose studies, limited/no controlled studies in prolonged users Not all possible adulterants tested, particularly those effecting the pH of the sample Oral ingestion/hygiene and drug exposure duration can affect results
Blood	0–48 h	Only specimen that is accepted by courts of law for ethanol	Medical professional needed for collection Invasive Usually have to rely on detection of parent compound, metabolites usually of minimal benefit
Urine	0–96 h Up to 30 d for THC	Most-established sample type No need for medical professional	Requires restroom for collection Can be adulterated/tampered with
Hair	1–12 mo	Ability to detect ingestion for up to 1 y	Extensive sample preparation needed by laboratory Testing not available immediately Can have higher amount of false positives if sample preparation done incorrectly Extensive training required for collector Possible hair damage from chemicals used for styling and/or grooming

Abbreviation: THC, tetrahydrocannabinol.

Chromatography with Tandem Mass Spectrometry (LCMS/MS) confirmatory analysis, using either multiple transitions or 2 different prepared samples. The LCMS/MS is significantly more sensitive than immunoassay and, thus, will potentially be able to detect substances that are less than immunoassay cutoffs.

Thus, it is not uncommon for a clinician to question the laboratory when a patient's urine results as negative using the screen (Immunoassay [IA]) technology and positive (ie, 156 ng/mL) on confirmatory (LCMS/MS) technology, often performed by 2 different laboratories, thereby casting doubt on the proficiency of one laboratory over another. This is erroneous, because in such a case it could be that the IA screen lower limits of detection (LOD) could be 300 ng/mL, whereas the LCMS/MS confirmatory LOD could be 50 ng/mL, thus explaining the negative screen and positive confirmation of a concentration of 156 ng/mL, in addition to other interfering factors that can affect the IA result.

Therefore, it is the responsibility of the provider to know what testing platforms are used at the laboratories and what the differences are between the laboratories before making decisions on the results.^{2,14}

Interpretation

The toxicology result is often called on to render a decision of whether patients are taking their prescribed medications, taking other nonprescribed medications, and/or illicit substances. Although the detecting technologies and the inherent interferences have been discussed, there is also another layer of interrogation and thought that needs to be manifest to interpret a toxicology laboratory result. The laboratory's purpose is to detect or not detect and, where applicable, provide a concentration of the analyte. It is not the position of the routine (vs forensic) clinical toxicology laboratory to render a unilateral decision of whether patients diverted, surreptitiously administered, or overdosed on a medication or illicit substance. Such an assessment should be made in the context of integrating many other aspects of the patient-physician relationships (ie, track marks on the arm, "meth head" countenance, inconsistencies in patient history, and so forth). Indeed there have been reports of secondhand exposure to various narcotics, such as cocaine,^{15,16} marijuana,¹⁷⁻¹⁹ and other agents, through ventilation, passive exposure, skin, and other means,^{15,16,20,21} as well as ingestion of herbal remedies that were unknowingly tainted with analgesic and illicit substances, such as benzodiazepines and opiates, among others.^{22,23} A positive result may avail itself depending on which technology and which cutoff values are in place and can differ among laboratories. To this end, ElSohly¹⁵ reported the presence of cocaine in the urine after the handling of cocaine-laced money, with a peak concentration of 72 ng/mL over a 24-hour period. As stated in vignettes earlier in this review, such a concentration would test negative using screening immunoassay technology with a cutoff of 300 ng/mL, yet test positive with confirmatory (Liquid chromatography (LC) or gas chromatography (GC) MS/MS) technology with a cutoff of 25 ng/mL.¹⁵ Thus, the laboratory results can foster questions to explain inconsistencies rather than unilaterally vilify them.^{24,25}

SUMMARY

In terms of providing the accurate final result, a false-positive screening testing is not a concern for a toxicology laboratory that routinely provides screen tests that reflex to confirmation tests. However, false-positive screening results become a significant problem when confirmation testing is not offered, performed, or requested. The laboratory can only perform testing that is ordered by a client, be that a physician or other non-health care entity (human resources department, hiring agency, and so forth). If the ordering

entity chooses to save money by not requesting mandatory confirmation testing, those parties should be aware of the false-positive rate for all drug classes they are screening for.¹ Such false-positive results have resulted in confusion as well as employment termination and legal proceedings, which have been rectified with appropriate confirmations studies and better understanding of toxicology testing in general. Ordering entities should also be aware of the cross-reactivity rates of the screening tests they are ordering or using. Point-of-care testing units, such as all-in-one cup tests or other dipstick-type testing device, have been documented as having significantly higher cross-reactivity rates and, thus, higher false-positive rates than laboratory-run immunoassay tests. Additionally, the same precautions should be evaluated if an ordering entity does not request all positive screening tests be sent for confirmatory testing. In conclusion, it is the ordering entities' responsibility to understand the advantages and limitations of the drug testing they are ordering and use such objective ancillary results in the context of other information obtained during the clinical assessment to support and facilitate appropriate clinical management. The clinical toxicology laboratory can be an invaluable asset²⁶ in facilitating understanding and education in assisting the clinician with respect to laboratory testing and resulting to achieve this end point.

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