Analytical Testing Methods

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Background

The Mandatory Guidelines for Federal Workplace Drug Testing Programs require a laboratory to conduct two analytical tests before a urine specimen can be reported positive for a drug, adulterated, or substituted. The first and second tests are generally referred to as the initial and confirmatory tests, respectively. Specifically, for drug analyses, these tests are called initial drug and confirmatory drug tests. The tests that are conducted to determine if a urine specimen is adulterated or substituted are specifically called initial validity and confirmatory validity tests.

Initial Drug Tests

An initial drug test (also known as screening test) is defined as an immunoassay test used to eliminate "negative" urine specimens from further consideration and to identify the presumptive positive specimens that require confirmation or further testing. Generally, every urine specimen received by a laboratory is screened for the five drug classes (i.e., amphetamines, cocaine, opiates, phencyclidine, and marijuana) as described in the Mandatory Guidelines unless a Federal agency chooses to only test its specimens for marijuana and cocaine. To test for five drug classes, a different initial (immunoassay) drug test kit is used for each drug class (i.e., five drug classes – five different initial test kits). Each drug test kit used by a laboratory must be approved for commercial distribution as an in vitro diagnostic test by the Food and Drug Administration (FDA). A number of different immunoassay techniques are available to screen urine specimens for the five drug classes. These techniques include radioimmunoassay (RIA), enzyme immunoassay (EIA), kinetic interaction of microparticles in a solution (KIMS), cloned enzyme donor immunoassay (CEDIA), and fluorescence polarization immunoassay (FPIA).

The basic principle behind each immunoassay technique relies on the competitive binding of drug (that may be in a specimen) and a labeled drug to an antibody. Antibodies are proteins that chemically bind with specific substances called antigens (i.e., a drug or drug metabolite). In an immunoassay test, a known amount of an antibody is added to the urine specimen. In addition, a known amount of labeled drug or drug metabolite (antigen) is added to the specimen. Any drug or drug metabolite present in the specimen will compete with the labeled drug or metabolite to bind with the antibodies forming antigen-antibody complexes. The amount of labeled antigen that is able to bind with an antibody is a function of the amount of drug or drug metabolite in the urine. Spectrophotometric endpoints of these reactions are used to semi-quantitatively identify drugs and/or drug metabolites in each urine specimen.

As specified in the Mandatory Guidelines, there is an initial test cutoff concentration that is used for each drug class to call a urine specimen negative or positive. Based on the cutoff concentration used for each different drug class, a negative specimen is any specimen that

contains no drug or whose apparent concentration of drug or drug metabolite is less than the cutoff concentration used for that drug or drug class.

Since immunoassay tests may have some cross-reactivity with drugs or drug metabolites other than the drug or drug metabolite for which it is primarily testing for, some laboratories may use a second initial drug test (different than the first initial drug test) prior to conducting a confirmatory test in an effort to enhance the specificity of the immunoassay. If a laboratory uses a second initial drug test to further identify a urine specimen as presumptive positive prior to conducting a confirmatory test, the second initial drug test is subject to the same criteria and requirements as the first initial drug test.

Confirmatory Drug Tests

A urine specimen that is presumptive positive for a drug or drug metabolite on an initial drug test must be tested a second time to confirm the presence of the drug or drug metabolite. The laboratory must conduct the confirmatory test on a second aliquot (i.e., a small amount of urine) that is removed from the original specimen bottle. The requirement to use a second aliquot ensures that the results for the two tests are for the same urine specimen. The Mandatory Guidelines require that gas chromatography/mass spectrometry (GC/MS) be used as the confirmatory drug test to confirm a presumptive drug positive result.

In general, a confirmatory drug test includes selectively extracting a drug or drug metabolite from a urine specimen and concentrating the drug or drug metabolite into a small volume (e.g., 0.5 mL or less) of an appropriate solution. The extraction procedure is necessary to remove the drug or drug metabolite of interest from other "interfering" substances present in urine. Once the drug or drug metabolite has been isolated and concentrated into an appropriate extract using a solid phase or solvent-solvent extraction procedure, the extract is injected into a GC/MS. GC/MS is actually a combination of two different analytical techniques. Gas chromatography relies on physically separating the drug or drug metabolites in the extract from one another as they pass through a long, small diameter column. The drug or drug metabolites migrate at different rates along the column and, therefore, exit the column at different times before passing through a detector. The time it takes for a drug or drug metabolite to pass through the column is called the retention time and when compared to a standard gives a preliminary identification of a drug or drug metabolite.

For regulated drug testing programs, a mass spectrometer must be used as the detector because it can positively identify a particular drug or drug metabolite. A mass spectrometer converts a drug or drug metabolite into charged particles and the mass-to-charge (m/z) ratios of the particles generated create a pattern that provides a positive identification of the drug or drug metabolite at the measured retention time compared to a standard. The two types of mass spectrometers used by laboratories for testing regulated specimens are either electron ionization (EI) or chemical ionization (CI) mass detectors. EI bombards a drug or drug metabolite molecule with a stream of high energy electrons, thereby, creating a number of fragment ions in a pattern that is unique to a particular drug or drug metabolite. The EI can be operated in a full-scan mode (all ions are tracked for a given scan) or in a selected ion monitoring (SIM) mode (only pre-selected ions are

tracked for a given scan). The SIM mode is the most widely used mode by the drug testing laboratories because the mass spectrometer is collecting data on only the pre-selected ions which improves the sensitivity of the method. When the ratios of the pre-selected ions agree with the ratios for a known standard under the same experimental conditions, a drug or drug metabolite is positively identified and its concentration can be determined. In contrast, CI is considered a soft ionization technique that produces very few ions, but the intensity of the molecular weight ion fragment is most often the most intense peak. Rather than using a stream of high energy electrons, CI introduces a reagent gas into the mass spectrometer and a filament ionizes the reagent gas. The ions generated transfer a charge to the drug or drug metabolite. This can result in positive or negative charged fragments. As with EI, the fragment pattern of the drug or metabolite created by CI is compared to a standard under the same experimental conditions, thereby, providing a positive identification of the drug or drug metabolite.

Identification of a drug or drug metabolite relies on acceptable chromatographic separation, acceptable mass spectrometric information, and the data satisfying all quality control and calibrator requirements. The concentration of a drug or drug metabolite is calculated using either peak areas or heights for the mass ions when compared to a standard.

Initial and Confirmatory Validity Tests

The analytical methods used to determine the validity of a urine specimen are quite different from those used to test a urine specimen for drugs because of the variety of adulterants that may be used by donors to tamper with a specimen.

The following methods are among those that are used for conducting initial and confirmatory validity tests:

- **Colorimetry.** An analytical procedure based on comparison of the color developed in a solution of a test material with that in a standard solution and quantitated on the basis of the absorption of light. In a colorimetric test method, reagents are added to a sample of urine and a reaction occurs with the analyte of interest, producing a color. Because the intensity of the color is related to the analyte's concentration, the concentration of the analyte is determined by visually measuring the color or electronically measuring the intensity of light at selected wavelengths (i.e., spectrophotometry). Creatinine concentration and pH may be determined using colorimetric tests.

- **Refractometry.** A refractometer measures indices of refraction. The index of refraction is the ratio of electromagnetic radiation in a vacuum to its velocity in the medium of interest. A urine specific gravity refractometer is used to determine the amount of solute (i.e., urinary total solids) in the urine by measuring the index of refraction. An instrument manufacturer applies a formula to convert refractive indices to urine specific gravity values which are displayed by the refractometer. Certified laboratories are required to use refractometers that report and display specific gravity to four decimal places.

- **Potentiometry.** The measurement of the electrical potential difference between two electrodes in an electrochemical cell. A pH meter is one type of potentiometer that is used to determine the pH of a urine specimen.

- **Atomic Absorption Spectrophotometry (AAS).** A sample is vaporized in a flame or graphite furnace and the atoms absorb ultraviolet or visible light and make transitions to higher electronic energy levels. The analyte concentration is determined from the amount of absorption of specific wavelengths. Chromium (VI) is an adulterant that may be tested using AAS.

- **Electrophoresis.** A separation technique that is based on the mobility of ions in an electric field. Positively charged ions migrate towards a negative electrode and negatively charged ions migrate toward a positive electrode. Ions have different migration rates depending on their total charge, size, and shape, and can therefore be separated. Capillary electrophoresis (CE) is an electrophoretic method using a small-bore, fused silica capillary tube. The capillary tube allows the use of very high electric fields because the small capillaries efficiently dissipate the heat that is produced. Increasing the electric fields produces very efficient separations and reduces separation times. Some adulterants that may be tested using CE are nitrite and chromium (VI).

- **Gas Chromatography/Mass Spectrometry (GC/MS).** Described above. Some adulterants that may be tested using GC/MS are glutaraldehyde and pyridine.

- **Inductively-Coupled Plasma-Mass Spectrometry (ICP-MS).** A sample is introduced into a radio frequency (RF) induced plasma in the form of a solution, vapor, or solid. The temperature of the plasma may reach up to 6000 Kelvin at the center and 8000 Kelvin at its periphery. The high thermal energy and electron rich environment of the ICP results in the conversion of most atoms into ions. A mass spectrometer detects the ions at each mass in rapid sequence, allowing signals of individual isotopes of an element to be scanned. Some adulterants that may be tested using ICP-MS are chromium (VI) and halogens.

- **Ion Chromatography (IC).** A form of liquid chromatography that uses ion-exchange resins to separate atomic or molecular ions based on their interaction with the resin. Its greatest utility is for analysis of anions (negatively charged ions) for which there are no other rapid analytical methods. It is also commonly used for cations (positively charged ions) and biochemical species such as amino acids and proteins. Some adulterants that may be tested using IC are nitrite, chromium (VI), and halogens.

- **Multi-Wavelength Spectrometry (MWS).** A method that measures multiple wavelengths of light (or other electronic transmissions) to identify an analyte. The method generates corrected absorbance values that are related to the analyte concentration. Some adulterants that may be tested using MWS are nitrite, chromium (VI), halogens, and surfactants.

- **Characteristic Immunoassay Drug Test Responses.** These are exhibited by some adulterants using some reagents. This enables laboratories to develop criteria for initial drug test data to identify a specific adulterant (e.g., glutaraldehyde). If these responses are validated by a laboratory for a specific adulterant, the laboratory may accept the abnormal drug test readings as the initial test for that adulterant. The laboratory must validate the immunoassay tests for this use and must analyze required controls in the same batch (i.e., a control with the adulterant and a control without the adulterant) to document that the adulterant produces those test responses. For the confirmatory test, laboratories must use a definitive method for identifying the adulterant (e.g., GC/MS for glutaraldehyde).