National Laboratory Certification Program
DRUG TESTING MATTERS

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Validating Specimen Validity Testing for Urine and Oral Fluid Drug Testing

*This is the updated second part of a five-part **Drug Testing Matters** series on urine and oral fluid drug testing method validation, originally published in 2015. This part covers validating specimen validity testing (SVT) methods. The first part covered validating immunoassay methods; the third and fourth parts will cover validating mass spectrometry methods; and the fifth part will cover validation of oral fluid collection devices.*

Specimen Validity Testing Overview

To avoid laboratory detection of drug use, donors may attempt to defeat a drug test by substituting or altering the specimen collection. The specimen composition can be affected by consuming *in vivo* "detoxifying agents" or excess quantities of liquids before collection. Specimens can also be altered through the *in vitro* addition of substances during the collection.

Substitution is used to describe submission of a product in place of the donor's specimen. Adulteration is a term used to describe the *in vitro* addition of a substance that either destroys the drug or drug metabolite that may be present or otherwise interferes with the ability of the laboratory to test the specimen. Many substances can be used to adulterate a urine specimen, including common household products, commercial chemicals, and commercial products developed specifically for drug test specimen adulteration. Exhibit 1 lists examples of contemporary "detoxifying agents," substitution products, and adulterants aimed at affecting the outcome of a urine drug test.

Exhibit 1. Examples of Commercial Products to Subvert a Drug Test

Product Name	Preparation	Classification
Clear Choice Quick Luck	Powdered	Synthetic Urine
Dr. John's Pee Pee	Pre-Mixed	Synthetic Urine
Spectrum Labs Quick Fix Plus	Concentrate	Synthetic Urine
TestClear Urine Simulation with Powdered Urine Kit	Powdered	Synthetic Urine
Serious Monkey Business Monkey Urine	Powdered	Synthetic Urine
Serious Monkey Business Monkey Flask	Pre-Mixed	Synthetic Urine
The Wizzinator Golden Flask (ALS)	Pre-Mixed	Synthetic Urine
ALS The Golden Shower	Powdered	Synthetic Urine
X-Stream	Pre-Mixed	Synthetic Urine
Rapid Clear Clean Pee	Concentrate	Synthetic Urine
Ultimate Gold	Pre-Mixed	Synthetic Urine
Safeguard Upass	Pre-Mixed	Synthetic Urine
Urine The Clear Full Dehydrated Urine Kit	Powdered	Synthetic Urine
Urine The Clear Full Frozen Urine Kit	Pre-Mixed, Frozen	Synthetic Urine
Ultra Klean Ultra Pure	Pre-Mixed	Synthetic Urine
Clear Choice Spike Additive	Single Solution	Adulterant
Spectrum Labs Urine Luck	Two Solutions	Adulterant
Klear Additive	Crystals	Adulterant

In general, immunoassay (IA) initial tests are more sensitive to the effects of adulterants than confirmatory tests. Depending on the adulterant and the IA reagent used, false-positive and false-negative drug test results may occur. The adulterant effects may be limited to a single IA drug class. Some adulterants affect the confirmatory test, which employs a more specific method (i.e., chromatography coupled with mass spectrometry) than IA. An adulterant may cause a false-negative or invalid result (1).

To detect donor attempts to manipulate specimens, drug testing programs rely upon SVT (2). As the numbers of suspect specimens increased over the years, the Department of Health and Human Services (HHS) developed and implemented SVT and reporting requirements for HHS-certified laboratories (3). "SVT" refers to a variety of tests that are used to determine if a urine specimen is:

- dilute,
- invalid,
- inconsistent with human urine (i.e., substituted), or
- adulterated.

The *Mandatory Guidelines for Federal Workplace Drug Testing Programs* and National Laboratory Certification Program (NLCP) Manuals include criteria for these reporting categories, along with other SVT requirements for federally regulated specimens (4, 5). Required urine SVT measurands include the following:

- Creatinine
- Specific gravity
- pH
- One or more oxidants

SVT is authorized but not required for oral fluid specimens. Laboratories must obtain Substance Abuse and Mental Health Services Administration (SAMHSA) approval before implementing an oral fluid specimen validity test for regulated specimens by notifying the NLCP and submitting supporting documentation for review.

Additionally, laboratories may conduct tests for biomarkers (i.e., endogenous substances used to validate a biological specimen) on a case-by-case basis as described in the *Mandatory Guidelines for Federal Workplace Drug Testing Programs using Urine* (UrMG) and the *Mandatory Guidelines for Federal Workplace Drug Testing Programs using Oral Fluid* (OFMG). Laboratories must submit assay validation records for biomarker assays for review prior to implementation.

Additional SVT analyses may be performed for a specimen that exhibits abnormal physical characteristics or drug testing results.

Urine Creatinine

Creatinine and specific gravity results are used to assess urine dilution and substitution.

Creatinine is a muscle creatine phosphate breakdown product that is an endogenous constituent of urine. Normal human urine creatinine concentrations are greater than or equal to 20 mg/dL. Abnormal creatinine results may be the effect of *in vitro* dilution of the specimen or *in vivo* effects of excessive fluid intake or renal disease (e.g., glomerulonephritis, pyelonephritis, renal failure). Consumption of meats may contribute to increases in urine creatinine. Evaluating urine creatinine values less than 20 mg/dL includes the results of specific gravity testing as discussed in the next section.

Creatinine is measured using the colorimetric Jaffé reaction method in which the creatinine and picric acid, in the presence of strong base such as sodium hydroxide (NaOH), combine to form a red-orange chromophore.

Urine Specific Gravity

Specific gravity (also known as relative density) depends on the concentration of the total dissolved particles in urine compared with water (water = 1.0000). Dissolved substances may include salts, glucose, protein, and urea. The normal range for human urine specific gravity is 1.0030 to 1.0200. Abnormal decreased urine specific gravity values may be the effect of *in vitro* dilution of the specimen or *in vivo* effects, including excessive fluid intake, renal failure, glomerulonephritis, pyelonephritis, or diabetes insipidus. Abnormal increased urine specific gravity values may result from *in vitro* adulteration or *in vivo* effects, including dehydration, diarrhea, excessive sweating, glucosuria, and water restriction.

Laboratories must perform specific gravity testing when the creatinine result is less than 20 mg/dL. Specific gravity may be measured using a 3-decimal place refractometer for screening when creatinine

is greater than 5 mg/dL and less than 20 mg/dL. A 4-decimal place refractometer must be used for specific gravity measurements when creatinine is less than or equal to 5 mg/dL or when the 3-decimal refractometer result is less than 1.002.

Urine pH

pH is tested to determine adulteration.

The value of pH is calculated as the negative base 10 logarithm of the hydronium ion activity in an aqueous solution. The acid or alkaline state of urine is controlled by the kidneys. The pH of normal human urine is usually near pH 7.0 (neutral) with a physiological range of approximately 4.5 to 9.0. Federal research has shown that summertime increases in specimen pH may be partially explained by a specimen's exposure to higher temperatures during storage and transport before arrival in the laboratory. Delays in transfer to refrigerated or frozen storage hastens bacterial growth, leading to increased pH. Urine pH less than 4.0 or greater than or equal to 11.0 is reported as adulterated.

Analytical methods for pH include examples such as dipsticks, pH paper, colorimetric (dye binding), and pH meter. A colorimetric pH test may be used as the initial pH test only if the test has a dynamic range of at least 3.0 to 12.0 and acceptable discriminatory capability at the cutoffs. The pH meter method is the only confirmatory pH method accepted for regulated testing. The other methods may be utilized for screening to determine whether the initial test is necessary.

Urine Oxidants

Oxidants are a class of chemicals that exhibit an oxidation-reduction ability to transfer oxygen atoms or accept electrons. The oxidant electron transfer process results in alterations to or destruction of molecular structure of drugs or drug metabolites. Oxidants may also affect immunoassay tests (by inhibiting enzymatic reactions) and confirmatory tests (when substances that compete for derivatizing reagents are present). Oxidants are not consumable and must be added to the urine *in vitro*. Common groupings of oxidants that may be encountered in drug testing include the following:

- Nitrite
- Chromium VI
- Halogens
- Pyridinium chlorochromate

Colorimetric oxidant methods of analysis may include general oxidants and nitrite-specific and chromium-specific colorimetry. Definitive analytical procedures for confirmatory or quantitative testing vary (see Exhibit 2).

Nitrite

The nitrite (NO_2) anion is an oxidizing agent produced from the reduction of nitrate (NO_3). Nitrites are found as active ingredients in commercial adulterant products and other commercial and naturally occurring sources. Normal human urine nitrite concentrations are less than 200 mcg/mL. The UrMG define specimens with nitrite levels greater than or equal to 200 mcg/mL and less than 500 mcg/mL as invalid and levels greater than or equal to 500 mcg/mL as adulterated. Drug measurements affected by the nitrite anion include marijuana metabolite, morphine, and the heroin metabolite (6-acetylmorphine). Urine nitrite concentrations will not exceed 200 mcg/mL for the following exposure sources:

- Foods (nitrite preservatives, nitrates in vegetables)
- Environmental run off contamination of drinking water
- Occupational exposure from explosives and pharmaceutical manufacture
- Medications for angina
- Human endogenous production
- Pathological production from infection/inflammatory conditions
- Medical pharmacotherapeutics such as interleukin-2 therapy
- Urinary tract infections.

Chromium VI

Chromium VI is an ingredient in commercial adulterant products and other commercial applications for chrome plating, dyes and pigments, leather tanning, and wood preserving. Chromium VI is toxic and has been shown to be a human carcinogen. Definitive confirmation of urine chromium VI greater than or equal to 50 mcg/mL is reported as adulteration.

Halogens

Halogen oxidizing agents include fluorine, chlorine, bromine, and iodine and their forms in which the halogen is combined with oxygen (e.g., hypochlorite, iodate, perchlorate, periodate). These oxidizing agents are ingredients in commercial adulterant products. None of the halogens are found in nature in their elemental form; however, elemental forms such as I_2 can be found in commercial health care products such as povidone-iodine, which is a common topical antibacterial (e.g., Betadine).

The SVT assays used by laboratories do not include halogen salts (e.g., NaCl, KCl), which may be present in a urine specimen. Definitive confirmation of urine halogens greater than or equal to the laboratory-defined limit of quantification (LOQ) is reported as adulteration.

Pyridinium Chlorochromate

Pyridinium chlorochromate is an ingredient in commercial adulterant products and other commercial applications for the preparation of medicines, vitamins, food flavorings, paints, dyes, rubber products, adhesives, insecticides, and herbicides. Definitive confirmation of urine pyridine greater than or equal to the laboratory-defined LOQ is reported as adulteration.

Other Urine Adulterants

Glutaraldehyde

Glutaraldehyde is an ingredient in commercial adulterant products and other commercial applications for sterilizing/disinfecting, leather tanning agents, tissue fixatives, embalming fluids, resins, or dye intermediates. Glutaraldehyde effects upon immunoassay testing vary according to the assay chemistry and the drug class. Definitive confirmation of urine glutaraldehyde greater than or equal to the laboratory-defined LOQ is reported as adulteration.

Surfactants

Surface-active agents (surfactants) are hydrophilic and hydrophobic molecules that reduce the surface tension of water when used in low concentrations. Examples include ordinary detergents, foaming agents, emulsifiers, and dispersants. Surfactants will form "bubbles" within the fluid: a small sphere of hydrophobic "heads" surrounding a pocket of air containing the hydrophilic "tails." SAMHSA has defined a cutoff level of 100 mcg/mL dodecylbenzene sulfonate equivalents. Surfactant activity is determined when results for two aliquots are ≥100 mcg/mL surfactant equivalents using a surfactant colorimetric test or a foam/shake test followed by a colorimetric test. Surfactant equivalents ≥100 mcg/mL are reported as adulteration only when a specific confirmatory test is performed.

Urine SVT Methods of Analysis

The method of analysis for an SVT depends upon the measurand(s). Examples include a wide variety of methods; see Exhibit 2.

Exhibit 2. SVT Methods of Analysis (1)

Method	Measurand	Description
Atomic Absorption Spectrophotometry (AAS)	Adulterant concentration (e.g., chromium VI)	An analytical method in which a sample is vaporized in a flame or graphite furnace. The atoms absorb ultraviolet or visible light at a specific wavelength and make transitions to higher electronic energy levels. The adulterant concentration is determined from the amount of absorption at the specific wavelength.
Capillary Electrophoresis (CE)	Adulterant concentration (e.g., nitrite, chromium VI)	An electrophoretic separation technique using a small-bore, fused silica capillary tube. This separation technique is based on the mobility of ions in an electric field. Positively charged ions migrate toward 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, which allows them to be separated.
Characteristic Immunoassay (IA) Drug Test Responses*	Adulterant concentration (e.g., glutaraldehyde)	Characteristic responses are exhibited by some IA tests in the presence of adulterants. This enables laboratories to develop criteria for initial drug test data that help identify a specific adulterant. If the IA response is validated by a laboratory for a specific adulterant, the laboratory may accept the abnormal drug results as the initial test for that adulterant. For the confirmatory test, laboratories must use a definitive method for identifying the adulterant (e.g., GC-MS for glutaraldehyde).
Colorimetry	pH, creatinine concentration, adulterant concentration (general or specific tests)	An analytical procedure based on comparison of the color developed in a solution of the tested material with that of a standard solution. In a colorimetric test, reagents are added to a sample and a reaction occurs, producing a color. Because the intensity of the color is related to the concentration of the measurand, the measurand is determined by visually measuring the intensity of light at selected wavelengths (i.e., spectrophotometry). This process is also used in some IA detection processes (e.g., ELISA).

Method	Measurand	Description
Gas Chromatography/ Mass Spectrometry (GC-MS, GC-MS/MS)**	Adulterant concentration (e.g., glutaraldehyde, pyridine)	An analytical method combining gas chromatographic separation and mass spectrometric measurements (*m/z* and abundance). Chromatographic separation and measurand retention times are based on gas-liquid partitioning of volatilized measurands. Examples include GC-MS and GC-MS/MS.
High-Performance Liquid Chromatography (HPLC)	Adulterant concentration (e.g., nitrite, chromium VI)	A chromatographic technique for separating and analyzing chemical substances in solution. Separation is based on absorption, partition, ion exchange, or size exclusion while the measurand remains in solution.
Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)	Adulterant concentration (e.g., chromium, halogens, surfactants)	An analytical method in which the 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 exceed 6,000K. The high thermal energy and electron-rich environment of the ICP results in the conversion of most atoms into ions. An MS is used to detect ions at different masses, allowing signals of individual isotopes of an element to be identified.
Ion Chromatography (IC)	Adulterant concentration (e.g., nitrite, chromium VI, halogens)	A form of liquid chromatography that uses ion-exchange resins to separate atomic or molecular ions based on their interactions with the resin. Its greatest utility in the federal program is for the analysis of anions for which there are no other rapid analytical methods. It is also commonly used for the analysis of cations and the separation of larger molecules, such as amino acids and proteins.
Multi-Wavelength Spectrometry (MWS)	Adulterant concentration (e.g., nitrite, chromium VI, halogens, surfactants)	A method that uses multiple wavelengths of light (or other electronic transmissions) to identify a measurand. The method generates corrected absorbance values that are related to the measurand concentration.
Potentiometry	pH, oxidizing adulterant concentration	The measurement of the electrical potential difference between two electrodes in an electrochemical cell. A pH meter is one type of potentiometer. The UrMG require certified laboratories to use a pH meter for the confirmatory pH test.
Refractometry	Urine specific gravity	The required test method for specific gravity analyses. A refractometer is used to determine the amount of solute (i.e., urinary total solids) in the urine by measuring the index of refraction. For program purposes, the index of refraction is the ratio of how much light is bent (refracted) by the urine sample. The instrument manufacturer applies a formula to convert from refractive indices to the urine specific gravity values displayed by the refractometer. Laboratories and instrumental initial test facilities (IITFs) may use refractometers accurate to 3 decimal places to determine whether an initial specific gravity test is needed. The UrMG require certified laboratories to use refractometers that report and display specific gravity to 4 decimal places for the initial and confirmatory specific gravity tests.

*See NLCP *Drug Testing Matters*, Validating Immunoassays for Urine and Oral Fluid Drug Testing.

**See NLCP *Drug Testing Matters*, Validating Gas Chromatography Mass Spectrometry for Urine and Oral Fluid Drug Testing.

The remainder of this publication focuses on validating SVT methods for initial tests and confirmatory tests.

SVT Validation[a]

Industry Standards

The method validation requirements described in this article are defined by HHS and the NLCP (4-7) for HHS-certified laboratories that test donor specimens in compliance with the UrMG and the OFMG. HHS-certified laboratories conduct forensic drug testing for federal agencies under Executive Order 12564 and Public Law 100-71 and for specific federally regulated industries. The *HHS Mandatory Guidelines for Federal Workplace Drug Testing Programs* affect all federal employees in a testing designated position, which is defined by each agency's Drug-Free Workplace Program.

Additional standards and guidance for forensic drug testing applications are published by the American National Standards Institute and the American Academy of Forensic Sciences Standards Board (ANSI/ASB) (8). The ANSI/ASB Standard 036 "Standard Practices for Method Validation in Forensic Toxicology" publication defines forensic toxicology validation practices, such as consensus standards, practice, and protocols, including quality assurance and quality control.

For Clinical Laboratory Improvement Amendments (CLIA) certification of clinical laboratories, the Centers for Medicare & Medicaid Services (CMS) requires laboratories to verify or establish performance specifications for any test system used by the laboratory on or after April 24, 2003 (9).

The goal of validating SVT methods is to provide objective data that (1) demonstrate that the method performs according to its intended use and (2) establish the method limitations under normal operating conditions.

Prior to implementation for use with testing donor specimens, all laboratories are required to validate the performance specifications of new methods, new instrumentation, and modifications to existing methods or instrumentation. Some laboratories perform supplemental SVT assays (e.g., to eliminate cross-reacting compounds, when the desired specificity cannot be obtained using the primary method). These supplemental SVT assays are subject to the same method validation requirements for the primary SVT assays.

An HHS-certified laboratory must demonstrate and document the appropriate performance characteristics of the test and must re-verify the test periodically, or at least annually, for each SVT assay except for pH and specific gravity. Each new lot of reagent must be verified prior to being placed into service.

Performance characteristic measurements include the following:

- The ability to differentiate valid specimens from those requiring further testing,
- The precision and accuracy of the test around the cutoff(s),
- The effective linearity of the test,

[a] *For clarity, this series of articles will use the term "validation" to cover all aspects of laboratory methods performance assessments, including verification of unmodified Food and Drug Administration (FDA)-cleared and FDA-approved assays and validation of laboratory-developed assays.*

- The potential for carryover,
- The specificity and potential for interfering substances, and
- Objective criteria to support characteristic immunoassay drug results as initial SVT results.

Documentation

SVT validation studies must be organized in a format that facilitates record review. Study records must include sufficient information to allow for a comprehensive review of the studies that were performed. Laboratories must describe criteria for acceptance of the validation study data, agreement of replicate study samples, and for defining or excluding true outlier values in both the study summary and the laboratory's standard operating procedures (SOPs).

At a minimum, the study records must include the following components:

- A stated purpose for the validation,
- Description of test methods,
- Identity of the instrument(s) used for the study,
- A listing of the instrument parameters used for the study,
- Description of the study samples,
- Summary of the statistical data collected to characterize the assay,
- A discussion,
- A summary with conclusions, and
- All raw analytical data from the samples analyzed in the study.

The laboratory must maintain the SVT validation study records for an indefinite period. Records for validation studies performed within the last 12 months must be available for review during NLCP inspections.

Each "end-user laboratory" must perform the validation and periodic re-verification studies for its assays and instruments. Off-site validations performed by other entities (e.g., manufacturer, other laboratory) may be used only to provide additional documentation.

Types of Validations

The types of validation studies to be used depend on whether the laboratory is implementing a new test, a new instrument model, or an additional instrument of the same model. Examples of validations include Assay, Full instrument, Abbreviated instrument, and Re-verification.

Note: Where multiple instrument models are used for an SVT assay, using the most conservative performance limits that can be determined for all models is acceptable, provided that this approach is described in the SOP and the validation study summaries.

SVT Assay and Full Instrument Validations

Validation of the SVT must be performed prior to implementing a new manufacturer's SVT reagent or when modifying an existing method. A change in the calibration scheme is an example of method modification. The same studies must also be performed for a full instrument validation, *prior to* implementing a new model of an instrument for use with a validated SVT. Where an SVT is used across multiple models of instruments, it is acceptable to define the performance for that SVT using the most conservative performance limits that can be determined for all of the models, provided that this approach is described in the SOP and the validation study summaries.

Required Studies

Creatinine tests, general oxidant colorimetric tests, and colorimetric tests for a specific compound with a program-defined cutoff

- Linearity and determination of the LOQ and upper limit of linearity (ULOL)
- Precision and accuracy around each cutoff
- Carryover
- Specificity/interference

Specific gravity and pH

- Precision and accuracy around each cutoff
- Carryover

Colorimetric tests (specific and general, both *without* a program-defined cutoff) and quantitative confirmatory tests for compounds

- Linearity, including LOQ and ULOL
- Carryover
- Specificity/interference

Colorimetric tests (specific and general, both *with* a program-defined cutoff) and quantitative confirmatory tests for compounds

- Linearity, including LOQ and ULOL
- Precision and accuracy around each cutoff
- Carryover
- Specificity/interference

Qualitative SVT (e.g., pH paper, pH dipstick, and differential dipstick assays; characteristic immunoassay drug test results indicative of an adulterant; foam/shake tests)

- Specificity/interference
- Studies to support objective test criteria established by the laboratory

Abbreviated Instrument Validation

An abbreviated instrument validation must be performed *prior to* implementing a new SVT instrument of a model type that has been validated by the laboratory. The studies required are the same as for re-verification studies of the assay. For pH and specific gravity assays, the same studies must be performed as for an assay or full instrument validation. The following studies are required:

Creatinine tests, colorimetric tests (specific and general, both *with* and *without* a program-defined cutoff), and confirmatory tests (both *with* and *without* a program-defined cutoff)

- LOQ and ULOL
- Carryover
- Specificity/interference

Specific Gravity and pH

- Precision and accuracy around each cutoff
- Carryover

Qualitative SVT (e.g., pH paper, pH dipstick, and differential dipstick assays; characteristic immunoassay drug test results indicative of an adulterant; foam/shake tests)

- Specificity/interference

Re-verification Studies

Re-verification studies must be performed annually for some SVT, as follows:

Creatinine tests, colorimetric tests (specific and general, both *with* and *without* a program-defined cutoff), and confirmatory tests (both *with* and *without* a program-defined cutoff)

- LOQ and ULOL
- Carryover
- Specificity/interference

Qualitative SVT (e.g., pH paper, pH dipstick, and differential dipstick assays; characteristic immunoassay drug test results indicative of an adulterant; foam/shake tests).

- Specificity/interference

Special Considerations for SVT Validation Studies

Cutoff(s)

Some SVT methods have more than one cutoff. SVT validation studies must include appropriate samples to evaluate the assay performance around each cutoff.

Carryover Studies

Carryover studies for creatinine, pH, and specific gravity must include appropriate samples to characterize the potential for carryover from a sample with low and high concentrations to another during testing. Decreased and increased results for these SVTs could adversely affect the donor. Laboratories must not set a carryover limit higher than the ULOL for creatinine.

Matrix Effects Studies

Some SVT methods may be susceptible to matrix effects (e.g., ionic strength may affect some colorimetric pH tests). The SVT validation studies should evaluate whether the test method is susceptible to matrix variations.

Assay-Specific Validation Notes

Creatinine

- Creatinine has 2 cutoffs: 2 mg/dL and 20 mg/dL.
- ULOL must be at least 200 mg/dL.
- Carryover to another sample must include a sample with no creatinine and a sample with high creatinine (at least as high as the ULOL).

pH Screening Test (pH paper or dipstick tests)

- After the original validation, each new lot of pH paper and dipsticks must be checked against the previous lot.
- Laboratories must document analysts performing these tests are able to distinguish color differences.

pH (other than pH paper or dipstick tests)

- pH has 4 cutoffs: 4.0, 4.5, 9.0, and 11.0.
- Colorimetric pH test must have a dynamic range of pH 3.0 to pH 12.0 to serve as an initial test.
- Some colorimetric pH screening assays may be impacted by samples with low ionic strength. Validation studies for pH screening assays should include a sample with low ionic strength. If the assay is affected, the laboratory must develop additional criteria to ensure that specimens with low ionic strength are reflexed for pH meter testing.
- Carryover assessment from one sample to another sample must be performed for all methods (colorimetric pH and pH meter) to document that carryover does not occur from a sample with low pH (less than pH 4.0) and from a sample with high pH (at least pH 11).
- Validation of a colorimetric pH test must include samples with a pH <1.5 to demonstrate that these samples would be reflexed to pH meter testing.

Specific Gravity

- 4-decimal place specific gravity has 3 cutoffs: 1.0010, 1.0030, and 1.0200.
- 3-decimal decimal place specific gravity has 1 cutoff: 1.002.
- Carryover assessment from one sample to another sample must be performed to document that carryover does not occur from a sample with low specific gravity (1.0000) and from a sample with high specific gravity (>1.0200).

Nitrite and Chromium VI

- Nitrite has 2 cutoffs: 200 mcg/mL and 500 mcg/mL.
- Chromium VI has 1 cutoff: 50 mcg/mL.
- Specificity studies should include urine with blood and chromium III in addition to other oxidants.

General Oxidant Screening Tests

- If the assay is used for
  - nitrite screening, 2 cutoffs using nitrite: 200 mcg/mL and 500 mcg/mL
  - chromium VI screening, 1 cutoff using chromium VI: 50 mcg/mL

Halogens, Glutaraldehyde,* Pyridinium Chlorochromate

- SAMHSA has not defined cutoffs; refer to laboratory-defined LOQ.

  **Note: Characteristic immunoassay assay response may be used for the glutaraldehyde initial test or to report a specimen as invalid–possible aldehyde activity. To validate the use of characteristic immunoassay drug test results, the laboratory must analyze samples with varying concentrations of the compound of interest and evaluate the immunoassay response for each drug channel. The laboratory is not required to perform a specificity study for this test.*

Surfactants

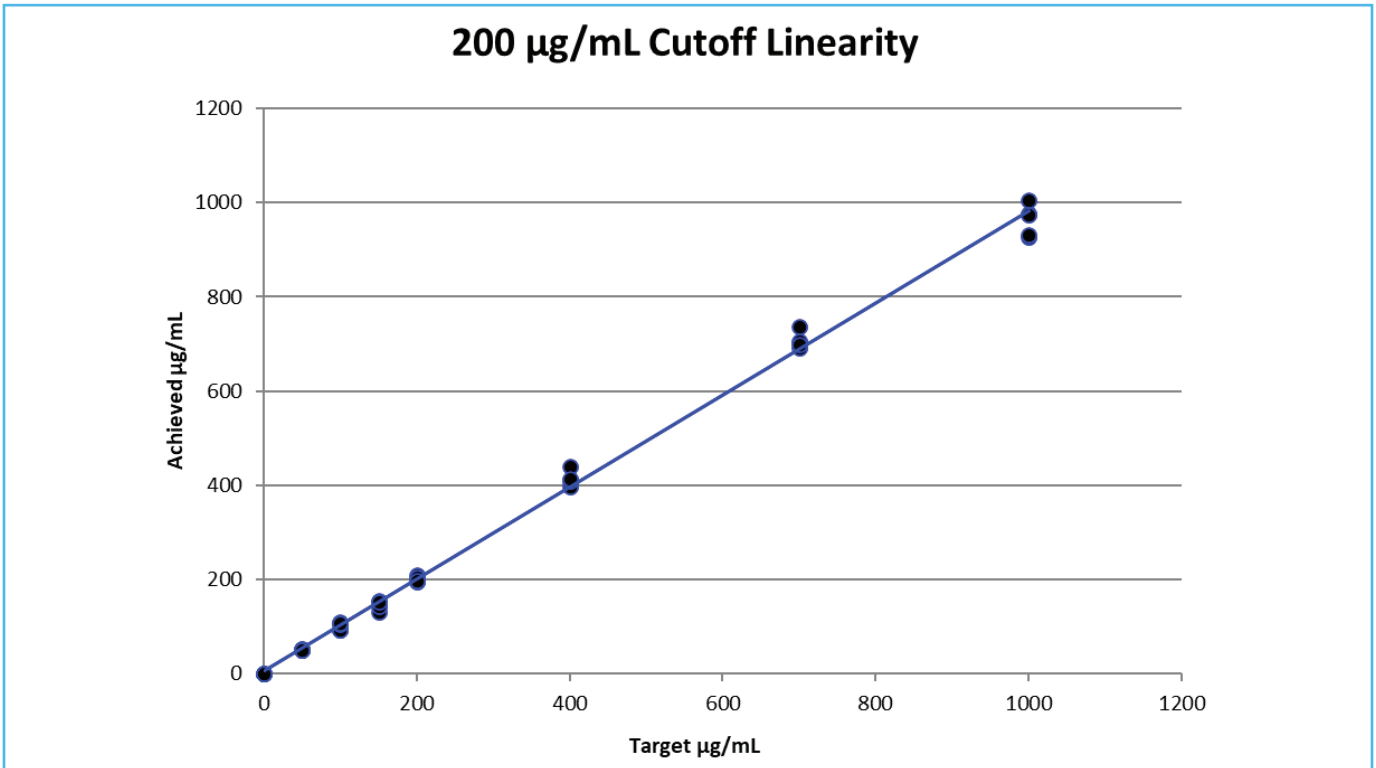
- Qualitative foam/shake tests must be witnessed by at least two individuals, including the certifying scientist.
- To validate a foam/shake test for surfactants, the laboratory must assess foaming in normal human urine and in urine with varying concentrations of surfactants and proteins added.
- The colorimetric surfactant test cutoff is 100 mcg/mL dodecylbenzene sulfonate equivalents.

Linearity Studies

SVT analyte concentrations should be distributed above and below each cutoff. SVT linearity study plots may be linear or non-linear. The low end of the regression curve or line represents insufficient measurand concentration for assay reaction or instrument measurement capability, whereas the upper end represents saturation of the assay reaction or instrument measurement capability. The laboratory must determine the linear portion of the response curve using at least five replicates for each of at least seven concentrations of the calibrator measurand.

The LOQ is the lowest concentration at which identity and concentration of the measurand can be accurately established. The ULOL is the highest concentration at which identity and concentration of the measurand can be accurately established. Values extrapolated from data or those that do not meet assay acceptance criteria may not be used for either LOQ or ULOL.

Exhibit 3. Example of SVT Linearity Study



Target µg/mL	Linearity 200 µg/mL Cutoff					Results			LOD LOQ ULOL
	1	2	3	4	5	Mean	−2SD	+2SD	
0	0.4	0.2	0.2	0.5	0.3	0.3	0.1	0.6	
50	49.9	51.7	49.9	53.1	50.8	51.1	48.4	53.8	LOD, LOQ
100	93.8	92.2	105.6	108.7	106.9	101.4	85.8	117.1	
150	132	146	144	154	152	146	128	163	
200	197	209	195	204	198	201	189	212	
400	439	397	412	409	415	414	384	445	
700	738	704	692	707	698	708	672	743	
1,000	977	1,006	974	926	932	963	896	1,030	ULOL

The linearity results must be plotted for review (e.g., for an SVT validation: instrumentation response value/units [Y axis] against concentration [X axis]).

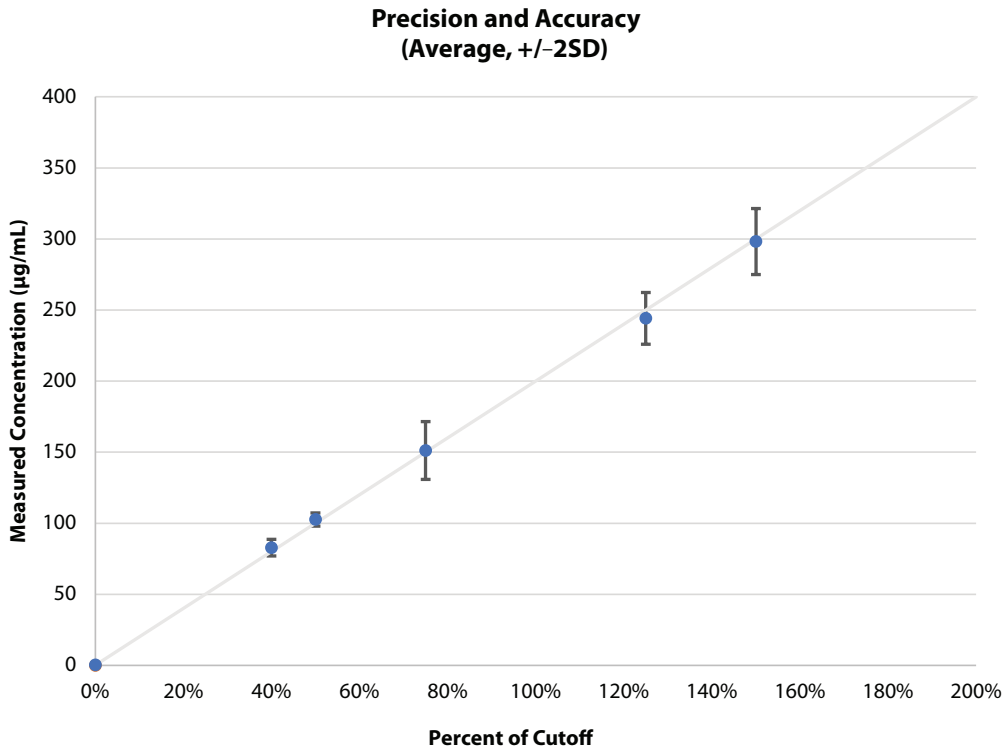
Precision and Accuracy

The laboratory must characterize the precision of the SVT using at least five replicates of calibrator measurand at critical concentrations relative to the cutoff. The concentrations relative to the cutoff should be distributed above and below each cutoff.

The precision data are evaluated by calculating the mean, standard deviation (SD), and the coefficient of variation (CV). The study samples must exhibit the appropriate response relative to each other (i.e., study samples should yield appropriate responses versus the cutoff and to that of the other study samples).

There should be no overlap of the 2SD ranges for the results of samples above and below each cutoff.

Exhibit 4. Example of SVT Precision and Accuracy Study



Precision and Accuracy 200 µg/mL Cutoff				
Target µg/mL	**Reps**	**Result µg/mL**	**Accuracy %**	**Precision**
0 µg/mL *0% of cutoff*	1 2 3 4 5	0.4 0.2 0.2 0.5 0.3	N/A	SD: 0.1 µg/mL
80 µg/mL *40% of cutoff*	1 2 3 4 5	84.2 86.3 83.5 81.1 78.7	+5.3% +7.9% +4.4% +1.4% −1.6%	SD: 2.9 µg/mL CV: 3.5%
100 µg/mL *50% of cutoff*	1 2 3 4 5	105.7 99.8 101.6 102.0 104.0	+5.7% −0.2% +1.6% +2.0% +4.0%	SD: 2.3 µg/mL CV: 2.2%

Precision and Accuracy 200 µg/mL Cutoff				
Target µg/mL	**Reps**	**Result µg/mL**	**Accuracy %**	**Precision**
150 µg/mL *75% of cutoff*	1 2 3 4 5	164 157 152 145 138	+9.3% +4.7% +1.3% −3.3% −8.0%	SD: 10 µg/mL CV: 6.7%
250 µg/mL *125% of cutoff*	1 2 3 4 5	260 240 237 241 243	+4.0% −4.0% −5.2% −3.6% −2.8%	SD: 9 µg/mL CV: 3.7%
300 µg/mL *150% of cutoff*	1 2 3 4 5	293 293 319 292 294	−2.3% −2.3% +6.3% −2.7% −2.0%	SD: 12 µg/mL CV: 3.9%

The laboratory must also characterize the accuracy of the assay by comparing each sample result with the target concentrations. It is acceptable to use the data from the precision study analysis for the accuracy study.

The precision and accuracy studies may be performed using separate batches on multiple days to characterize the intra-batch and inter-batch variability.

The laboratory must establish criteria for evaluating the statistical analysis. These must be described in the SOPs and the validation study summary.

Specificity

The laboratory is required to characterize the SVT performance when challenged with compounds that are commonly encountered in the testing population. Whether the presence of a compound would reduce (or increase) the response of the target analyte is of concern. The laboratory should consult peer-reviewed articles and reagent manufacturer information to identify compounds that may yield similar responses with the method used.

For SVT assays that identify invalid specimens (e.g., a nitrite-specific colorimetric test to report "invalid"), SVT specificity validation studies should include substances (e.g., endogenous compounds, food items, medications) that may give a test result meeting "invalid" criteria. The laboratory must evaluate potentially interfering compounds to determine the minimum concentration that produces an adulterated or substituted or invalid specimen result or, alternatively, to document that a very high concentration of the compound does not produce such a response.

For example, general oxidant test or specific oxidant colorimetric test SVT validation studies should include various oxidants' responses.

At a minimum, SVT specificity validation studies should include substances that may be present in human urine and that could cause an incorrect result that adversely affects the donor.

For example, creatinine SVT validation studies should include compounds that may lower the creatinine test result.

Some adulterants yield characteristically abnormal immunoassay responses. The laboratory should configure the analyzer to allow detection of immunoassay depression and should establish criteria for identifying invalid specimens based on the study results.

Carryover

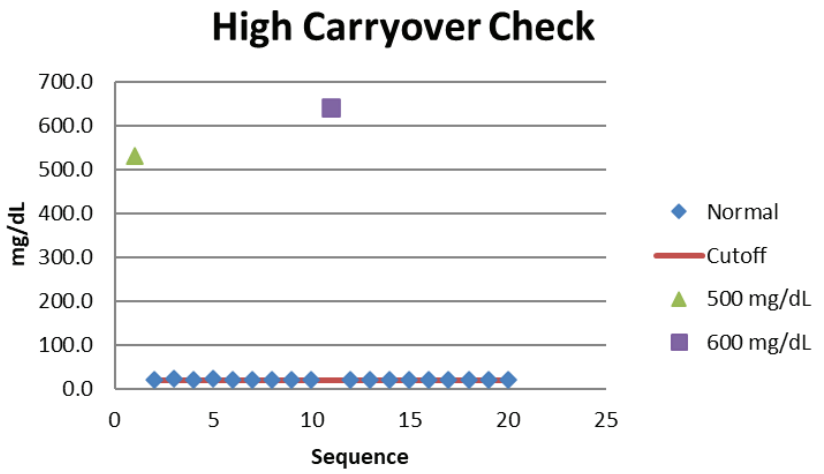
The laboratory is required to characterize the potential for carryover from one sample to another during testing. The laboratory should perform the carryover studies by analyzing highly concentrated samples followed by negative samples (i.e., without the analyte of interest) and evaluate the negative samples for carryover. The measurand concentrations in the high samples should be realistic (i.e., high concentrations that may be found in the testing population) and at least as high as the established ULOL. It is not acceptable for the laboratory's carryover limit to exceed the ULOL for an assay (e.g., creatinine, oxidants). Although the actual carryover limit may be greater than the ULOL, operationally, the laboratory cannot rely on quantifications greater than the ULOL. Therefore, the laboratory must use either the ULOL value or administratively set a value lower than the ULOL as the prompt for corrective action.

Creatinine, pH, and specific gravity SVT assays also require carryover studies analyzing samples with low concentration/values followed by samples with analytes of interest. If the laboratory does not have an established carryover limit for their pH and specific gravity tests (i.e., no carryover after the values tested

during the validation), then the laboratory should document the highest value (and the lowest value for pH) tested during the original validation as the carryover limit.

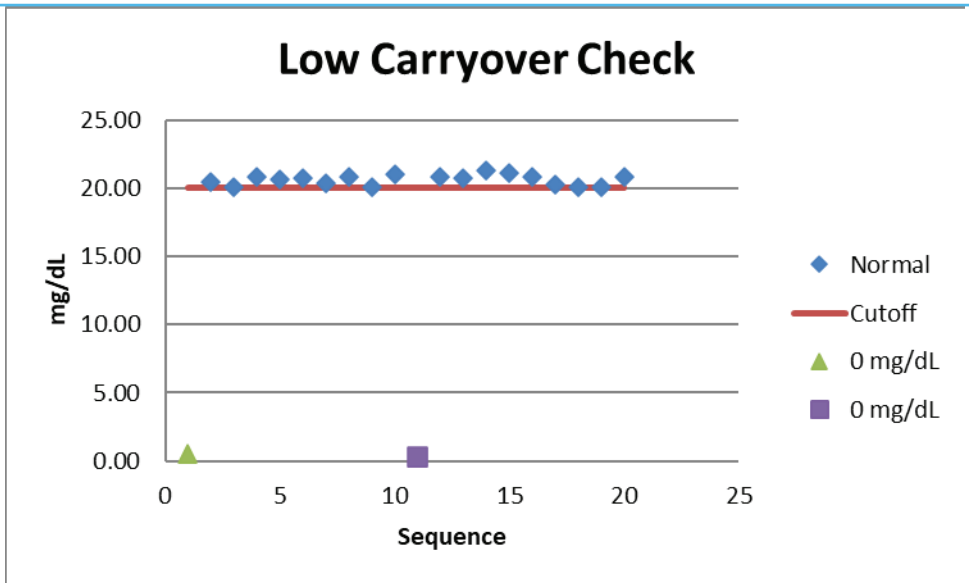
The laboratory must establish criteria (i.e., allowable response or concentration) for evaluating a sample tested after a sample that exhibited a value above the ULOL or carryover limit as appropriate. These must be described in the validation study summary and in the SOP.

Exhibit 5. Example of SVT High Carryover Study



High Carryover Check Samples		Absorbance Values (AU)	
		Sample Result	Cutoff Calibrator
1	500 mg/dL creatinine	530.0	20.0
2	Normal Urine	21.0	20.0
3	Normal Urine	21.6	20.0
4	Normal Urine	20.7	20.0
5	Normal Urine	21.5	20.0
6	Normal Urine	20.9	20.0
7	Normal Urine	20.8	20.0
8	Normal Urine	20.9	20.0
9	Normal Urine	21.3	20.0
10	Normal Urine	21.0	20.0
11	600 mg/dL creatinine	640.0	20.0
12	Normal Urine	20.8	20.0
13	Normal Urine	20.7	20.0
14	Normal Urine	20.6	20.0
15	Normal Urine	20.9	20.0
16	Normal Urine	20.5	20.0
17	Normal Urine	20.7	20.0
18	Normal Urine	20.9	20.0
19	Normal Urine	21.3	20.0
20	Normal Urine	21.0	20.0

Exhibit 6. Example of SVT Low Carryover Study



Low Carryover Check Samples		Absorbance Values	
		Sample Result	Cutoff Calibrator
1	0 mg/dL	0.50	20.00
2	Normal Urine	20.50	20.00
3	Normal Urine	20.10	20.00
4	Normal Urine	20.80	20.00
5	Normal Urine	20.60	20.00
6	Normal Urine	20.70	20.00
7	Normal Urine	20.40	20.00
8	Normal Urine	20.80	20.00
9	Normal Urine	20.10	20.00
10	Normal Urine	21.00	20.00
11	0 mg/dL	0.30	20.00
12	Normal Urine	20.80	20.00
13	Normal Urine	20.70	20.00
14	Normal Urine	21.30	20.00
15	Normal Urine	21.10	20.00
16	Normal Urine	20.80	20.00
17	Normal Urine	20.30	20.00
18	Normal Urine	20.10	20.00
19	Normal Urine	20.10	20.00
20	Normal Urine	20.80	20.00

Additional Notes

- It is usually necessary to perform complete validation studies for revised assays. However, if the modification is relatively minor, the validation studies may focus on parameters that may have been affected.

References

1. U.S. Department of Health and Human Services, Substance Abuse and Mental Health Services Administration, Center for Substance Abuse Prevention. Medical review officer guidance manual for federal agency workplace drug testing programs. 2020. Contract No.: Rev 0722. Available from: https://www.samhsa.gov/sites/default/files/2020-mro-manual.pdf
2. RTI International. Analytical methods in workplace drug testing immunoassay, National Laboratory Certification Program Training Courses. RTI International; n.d. Contract No.: available to NLCP inspectors and staff of HHS-certified laboratories.
3. U.S. Department of Health and Human Services, Substance Abuse and Mental Health Services Administration. Program Document 37: Notice to HHS-certified laboratories and inspectors, Subject: Specimen Validity Testing. 1999 July 28.
4. Mandatory Guidelines for Federal Workplace Drug Testing Programs using Urine (effective October 1, 2017), 82 FR. Sect. 7920-7970 (2017).
5. Mandatory Guidelines for Federal Workplace Drug Testing Programs using Oral Fluid (effective January 1, 2020), 84 FR. Sect. 57554-57600 (2019).
6. RTI International, Center for Forensic Sciences. Manual for Urine Laboratories, National Laboratory Certification Program (NLCP). Research Triangle Park, NC: RTI International, Center for Forensic Sciences; 2017. Contract No.: Rev. 0222.
7. RTI International, Center for Forensic Sciences Manual for Oral Fluid Laboratories, National Laboratory Certification Program (NLCP). Research Triangle Park, NC: RTI International, Center for Forensic Sciences; 2020. Contract No.: Rev. 0222.
8. ANSI/ASB Standard 036. Standard Practices for Method Validation in Forensic Toxicology. 2019. Available from: https://www.aafs.org/sites/default/files/media/documents/036_Std_e1.pdf
9. Clinical Laboratory Improvement Amendments (CLIA), Standards and Certification, Laboratory Requirements, Subpart K, Standard: Establishment and verification of performance specifications, 42 CFR. Sect. Part 493.1253.

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