New Second Generation Specimen Validity Testing for Urine Drugs-of-Abuse Testing

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Background

During the past decade, the percentage of positive urine drugs-of-abuse (DOA) tests has remained stagnant while the overdose death rate and the number of opioid doses have steadily increased. The subversion of urine DOA screening is a major problem. Due to ineffective pre-analytical subversion detection, the first generation of specimen validity tests (SVTs) can only detect 2 of 18 classes of subversion.

Objectives

A principal objective of the VDx GEN2-SVT™ panel is to increase the effectiveness of pre-analytical SVT testing to include 18 classes of subversion. Secondarily, the detection of subversion should be viewed as presumptive evidence of a substance use disorder (SUD). These objectives can be achieved without undue burden on laboratories by adding only two new tests to the current panel of SVTs commonly used on automated instruments.

Methods

The Validity Diagnostics (VDx) urine sample validity test (SVT) reagents are a second-generation urine SVT test panel. This unique set of reagents (VDx GEN2-SVT™ Panel) was developed to fill the gaps in the first-generation SVT reagents. The VDx improvements to the first-generation SVT reagents can be summarized as follows: **pH** - this reagent was improved by using two pH indicators instead of one indicator. The dual indicator reagent allows for very accurate readings at the low end (pH 3) and at the high end (pH 10.5). The VDx pH reagent can detect strongly buffered solutions. Urine **CREATININE** – this reagent was improved by add a decolorizing reagent to minimize interferences from hemoglobin. In addition, the linearity of the test was expanded past 250 mg/dL. Most importantly, it correlates with the SGI test below to detect creatine/protein loading of dilute urine samples. **Specific Gravity Index (SGI)** – this reagent is totally different from the classical specific gravity measurement. It uses an enzyme (beta-galactosidase) to measure total urinary sodium (Na+) and potassium (K+) ions.

If this enzyme is affected by adulterants, the adulterants may also affect the enzyme used in the enzyme immunoassay drugs of abuse (DOA) tests. The SGI test can also detect dilution and salting. **Oxidant History (OXH)** – this reagent measures (in uric acid equivalents) the effect of an oxidizing reagent by measuring the decrease in urinary levels of markers (e.g., uric acid, ascorbic acid, glutathione, cystine, cysteine, and phenolics). This effect can be measured for up to 30 days. Otherwise, using the first-generation SVT reagents, the adulterant (nitrite equivalents) may not be detected after 12 hours. **True Urine LD / SD (TRUU-LD/SD)** – these two tests in combination are used to detect three urinary tract proteins (UTP). The LD marker, UTP-IV, is from kidney tissue and has longer durability in urine. The SD markers (UTP-II & III) are short-lived markers arising from the urinary tract which makes it difficult to manufacture, ship, and store synthetic urine containing the markers. The selection of UTPs was optimized to make the effectiveness of the VDx panel more robust in detecting synthetic or substituted urine.

Results

The analysis of ~ 5,000 urine specimen results from several clinical laboratories using the VDx panel showed that ~ 10 - 20% of urine specimens had indicators of subversion and were considered “invalid” for subsequent urine DOA screens. This is in contrast to ~ 5% urine specimens showing subversion by traditional methods.

Among the invalid urine specimens, many were determined to be substituted or synthetic urine by both the True Urine LD (associated with long-duration protein detection) and SD (associated with short-duration protein detection) tests. Some of these urine specimens screened positive for the prescribed drug class. However, LC/MS-MS analysis confirmed the presence of a very large level of the parent drug but no metabolites of the parent drug. The presence of a large level of parent drug without metabolites could indicate “pill-shaving” or “drug-spiking.”

An example of “pill-shaving” involves a urine sample which screens presumptively positive for oxycodone but fails the True Urine LD and SD tests. The subsequent LC/MS-MS analysis of this urine sample yields a very high level of oxycodone but no oxymorphone or noroxycodone.

Conclusion / Discussion

When used in combination, the VDx GEN2-SVT™ panel increases the effectiveness of pre-analytical SVTs to include 18 classes of subversion commonly used by individuals with a substance use disorder. The 18 classes of subversion defined by VDx are:

1: Adulteration with strong acid or alkali,
2: Simple dilution,
3: Masked dilution (e.g., creatine/protein-loading),
4: Adulteration with salt,
5: Adulteration with oxidant,
6: Adulteration with glutaraldehyde,
7: Adulteration with heavy metal,
8: Substitution with synthetic urine,
9: Substitution with the urine of another person,
10: Substitution with freeze-dried human urine,
11: Adulteration with cationic detergent,
12: Adulteration with protease,
13: Adulteration with acid buffer pH > 3.0,
14: Adulteration with alkaline buffer < pH 10.5,
15: Substitution using a catheter,
16: Presence of enzyme inhibitor,
17: Fraudulent prescription acquisition with DOA-spiked synthetic urine, and
18: Adulteration with glucuronidase inhibitor.

The increased effectiveness can be achieved without undue burden on laboratories by adding only two new tests to the current panel of tests. Urine specimens passing the pre-analytical validity tests can undergo urine DOA screening with more confidence. Urine specimens from donors failing subversion testing by the VDx GEN2-SVT™ panel should be considered for testing by LC/MS-MS to broaden the number of drugs detected.

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