NOW YOU SEE IT, NOW YOU DON'T – THE CASE OF VANISHING DRUGS IN A URINE DRUG SCREEN

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Background

Urine samples are sometimes adulterated by persons when they are submitted for drug screening by enzyme immunoassay (EIA). Some means of urine sample adulteration can be detected by the first-generation urine sample validity tests (SVTs) but unscrupulous groups have invested significant amounts of time and money to sell products that defeat the first-generation urine SVTs. However, the second-generation (Validity Diagnostics - VDx GEN2-SVT™) urine SVTs can detect more classes of adulterants, including products that the donor consumes prior to submitting a sample for a urine drug screen that can mask the detection of drugs. The Validity Diagnostics GEN2-SVT™ reagent panel includes a dual-indicator pH reagent; a creatinine reagent with a decolorizing reagent to minimize interferences from hemoglobin; a specific gravity index reagent which measures total urinary sodium and potassium ions; an oxidant history reagent which measures the effect of an oxidizing agent; and, a reagent to detect urinary tract proteins.

Objectives

Identify potential sources of adulterants in "detox" drinks which may mask the detection of drugs by EIA. Evaluate the performance and applicability of second-generation urine SVTs for clinical and forensic use for high throughput SVT testing.

Methods

A coworker, legally using various drugs, volunteered to consume a "detox" drink and to donate urine samples over a period of time. This "detox" drink, Rescue Detox Ice Instant Cleansing Energy from Applied Sciences, allegedly contains B/C vitamins, sodium, potassium, protein hydrolysate, caffeine anhydrous, creatine, inulin, and several botanical extracts and is marketed to mask drug detection when using EIA drug screens. The coworker followed the directions included with the "detox" drink and did not consume anything else during the urine collection period. Urine drug screens and first and second-generation urine sample validity tests were conducted on the urine samples.

The Validity Diagnostics (VDx) urine sample validity test (SVT) reagents are a second-generation urine SVT panel. The VDx SVT panel consists of the following: **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). Urine **CREATININE** – this reagent was improved by add a decolorizing reagent to minimize interferences from hemoglobin. 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.

Results

After consuming the "detox" drink, urine samples were collected at various times and were screened for drugs by EIA and were subjected to SVTs:

TIME ZERO and **25 minutes** – Positive for amphetamines, cannabinoids and ethyl glucuronide

57 minutes – Positive for amphetamines and ethyl glucuronide

68 minutes – Positive for amphetamines

76 minutes and **118 minutes** – NEGATIVE (these two urine samples passed the first-generation urine SVTs but failed the second-generation urine SVTs)

193 Minutes and **261 minutes** – Positive for amphetamines, cannabinoids and ethyl glucuronide

SPECIMEN	PH	URINE CREAT	SGI	OXH	TRUULD	C/SGR	STANDARD SG	STANDARD UC	THC (Cutoff 50)	ETG (Cutoff 1000)	AMP (Cutoff 500)
Normal Sample (8:45am)	6.1	209.6	1.0118	138.4	507.9	2.7	1.025	199.8	157.4	2876.1	542.2
Sample 1 (9:10am / 25mins	6.3	198.9	1.0124	194.8	348.6	2.4	1.023	185.5	151.8	2717.1	1007.8
Sample 2 (9:42am / 57mins)	5.2	79.1	1.0028	65.6	113.7	4.2	1.005	75.4	35.5	1297	943.7
Sample 3 (9:53am / 68mins)	5.6	25	1.0026	5.5	32.7	1.4	1.003	26.2	0	479.2	510.2
Sample 4 (10:01am / 76mins)	5.6	20.1	1.0026	3.7	23.7	1.2	1.003	21.4	4.8	268.1	406.7
Sample 5 (10:43am / 118mins)	5.7	20.8	1.0029	2.9	26.4	1.1	1.003	22.1	-1.5	204.8	347.9
Sample 6 (11:58am / 193mins)	5.4	140.7	1.0518	41.7	116.4	0.4	1.013	137.5	80.5	1353.3	737.4
Patient Sample 7 (1:06pm / 261mins)	6.4	164.8	1.0386	62.7	144.3	0.6	1.02	150.3	111.9	1318.1	680

Conclusion / Discussion

This study was conducted to help determine the possible cause(s) of several failed urine SVTs, negative drug screens and follow-up positive LCMS results in a court-ordered probation drug testing program. The urine samples in question were flagged using the second-generation SVTs (VDx GEN2-SVT™) but were not flagged by the first-generation SVTs. After further investigation, the persons on probation informed the court that they were using the "detox" drink to pass the drug screens. The persons on probation also stated that while using drugs and the "detox" drink for several months, they did not experience any failed urine SVT tests when the laboratory was using the first-generation SVT tests. Based upon the studies in the probation drug testing program and the clinical drug testing program at my location, the use of the second-generations SVTs can increase the overall effectiveness of pre-analytical SVT testing by detecting many classes of adulterants. Because the second-generation SVT reagents can be used on automated clinical analyzers, they can be used without undue burden on drug testing laboratories. Urine specimens from donors failing SVTs with the VDx GEN2-SVT™ panel should have follow-up testing by LC/MS-MS.

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