

# EVALUATION OF XYLAZINE TEST STRIPS (BTNX) FOR DRUG CHECKING PURPOSES

DRUG CHECKING



**Purpose:** This report details validation experiments carried out to evaluate the use of xylazine test strips for drug checking purposes, as well as the results from performed studies.

**Background:** Xylazine is an alpha-2 adrenergic agonist and veterinary drug that has become an adulterant commonly encountered in the recreational drug supply, most commonly among opioid and “dope” samples (e.g., fentanyl, heroin). As an adulterant, xylazine elicits pharmacologically advantageous effects alongside fentanyl which can be described as synergistic or prolonging in nature. Xylazine exposure or ingestion can lead to profound sedation and altered mental status. Severe adverse effects of xylazine use include skin ulcerations or wounds. Chronic use of xylazine may result in physiologic dependence and withdrawal symptoms can arise when use is ceased. Since 2021, **more than 90%** of “dope” samples tested from Philadelphia, PA, have contained xylazine and fentanyl, with xylazine purity (or concentration) ranging from less than 5% to greater than 70%. An **increase** in xylazine content among these samples has also been observed over this time frame. Due to prevalence and continued geographical spread of xylazine-adulterated fentanyl across the United States, lateral flow immunoassay test strips have been developed as a new tool to allow for field-based drug checking to determine whether or not a sample contains xylazine (similar to the prior process and philosophy associated with fentanyl test strips). To this end, xylazine test strips were obtained and evaluated to determine their accuracy and efficacy.

**Methods:**

**Xylazine Test Strips (XTS):** XTS were provided for evaluation by **BTNX Inc.** (Ontario, Canada). The XTS had a specification cut-off of 1,000 ng/mL. The lot number of the batch was DOA2211187 (Exp. 11/2024). Due to the novelty of these strips, only one lot was available at the time of assessment for evaluation.

**Validation:** To assess performance, positive and negative controls were prepared in the laboratory and analyzed in replicates of five. An interference study was conducted with testing in triplicate. Blank matrix sources were obtained as negative controls. Positive controls were prepared using a xylazine analytical reference material. Interference controls were prepared using specified analytical drug standards.

**Authentic Samples:** Drug materials obtained through public health partnerships were tested in triplicate as part of this study. The drug materials were comprehensively analyzed by GC-MS and LC-QTOF-MS to confirm the presence of individual drug components and quantitative testing was pursued for xylazine, fentanyl, and other select analytes.

**Testing Protocol:** For validation experiments, drug solutions were prepared in water at specified concentrations and the XTS were used in accordance with the manufacturer’s guidelines. For authentic samples, a 5 mg micro scoop was used to aliquot the drug material into a simulated cooker and 5 mL of tap water was added. A secondary dilution of the solution in the cooker to 30 mL was performed, when necessary, if the initial positive result was deemed “suspicious” (e.g., positive XTS with a cocaine sample). This testing protocol was selected because it is the current, preferred method for using fentanyl test strips (FTS) per the Philadelphia Department of Public Health. Mimicking FTS and XTS testing as a single procedure was determined to be most advantageous and practical.

**Results:** The XTS cut-off was observed to be approximately 2,000 ng/mL. No false positives from various sources of water were discovered. With the exception of lidocaine, no other drugs were found to exhibit false positives (or interferences) with the XTS. Lidocaine consistently produced false positives at 10,000 ng/mL (equivalent to approximately 1.0% of drug from purity testing) with the XTS, while testing at 1,000 ng/mL was negative. Thirty-four authentic drug materials were tested using XTS, including “dope”, cocaine, “crack”, methamphetamine, and MDMA samples. The XTS accurately identified all samples that contained xylazine throughout the study; no false negative results were observed. As expected based on results from the interference study, cocaine and fentanyl samples cut with lidocaine produced false positive results (n=5); however, the secondary dilution (to 30 mL) was successful at ruling out false positive results for three samples.

**Conclusion:** The performance of the xylazine test strips was deemed acceptable for drug checking purposes, demonstrating high sensitivity (100%), specificity (85%), and precision (91%). Lidocaine was identified as an interferent which may lead to false positive results in the field.

**Future Studies:** Future studies will be explored, including continued testing of authentic samples, testing of additional XTS batch lots, added drugs for interference studies and macro-dose interference studies to test higher concentrations of drugs, and assessment of the test strips using mock and authentic urine specimens, among other research opportunities.

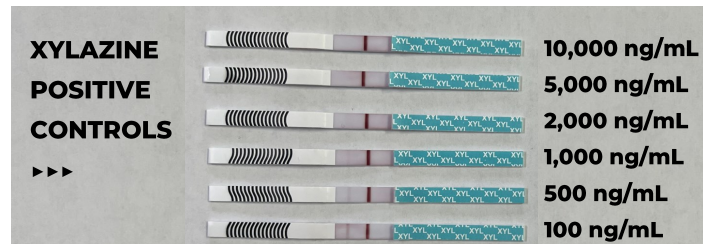
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NEGATIVE CONTROLS		
Sample	Concentration	Results (n=5)
Water (DI-1)	0 ng/mL	-----
Water (DI-2)	0 ng/mL	-----
Water (Tap-1)	0 ng/mL	-----
Water (Tap-2)	0 ng/mL	-----
Water (Tap-3)	0 ng/mL	-----
Water (Bottled-1)	0 ng/mL	-----
Water (Bottled-2)	0 ng/mL	-----
Water (Bottled-3)	0 ng/mL	-----
Water (Bottled-4)	0 ng/mL	-----
Water (Filtered)	0 ng/mL	-----

POSITIVE CONTROLS		
Sample	Concentration	Results (n=5)
Xylazine Standard	10,000 ng/mL	+++++
Xylazine Standard	5,000 ng/mL	+++++
Xylazine Standard	2,000 ng/mL	+ - + - +
Xylazine Standard	1,000 ng/mL	-----
Xylazine Standard	500 ng/mL	-----
Xylazine Standard	100 ng/mL	-----



INTERFERENCE CONTROLS		
Drug	Concentration	Results (n=3)
Acetaminophen	10,000 ng/mL	---
Benzocaine	10,000 ng/mL	---
Caffeine	10,000 ng/mL	---
Cocaine	10,000 ng/mL	---
Diphenhydramine	10,000 ng/mL	---
Lidocaine	10,000 ng/mL	+++
Lidocaine	1,000 ng/mL	---
Methamphetamine	10,000 ng/mL	---
Phenacetin	10,000 ng/mL	---
Procaine	10,000 ng/mL	---
Quinine	10,000 ng/mL	---
Theophylline	10,000 ng/mL	---

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## AUTHENTIC DRUG MATERIALS

#	Type	Sample Contents (% purity or respective parts, p)	Approximate Xylazine Conc.	Test 1: Results (n=3)	Test 2: Results (n=3)	XTS Results: Expected vs. Observed
1	Dope	Xylazine (23.4%), Fentanyl (21.0%), 4-ANPP (1.5%)	234,000 ng/mL	+++	+++	Positive / Positive
2	Dope	Xylazine (52.9%), Fentanyl (7.2%), 4-ANPP (0.7%), Bupropion (trace), Quetiapine (trace)	529,000 ng/mL	+++	+++	Positive / Positive
3	Dope	Xylazine (59.9%), Fentanyl (2.3%, 1p), <i>para</i> -Fluorofentanyl (1.3%), 4-ANPP (1.1%), Bupropion (0.2p), Quetiapine (0.2p)	599,000 ng/mL	+++	+++	Positive / Positive
4	Dope	Xylazine (10.1%), Fentanyl (3.7%, 1p), <i>para</i> -Fluorofentanyl (3.4%), 4-ANPP (0.9%), <i>para</i> -Fluoro-4-ANPP (0.6p), <i>para</i> -Fluoro-Phenethyl-4-ANPP (0.1p), Caffeine (0.7p)	101,000 ng/mL	+++	+++	Positive / Positive
5	Dope	Xylazine (35.5%), Fentanyl (7.6%, 1p), <i>para</i> -Fluorofentanyl (1.7%), 4-ANPP (2.1%), Phenethyl-4-ANPP (trace), Caffeine (0.2p)	355,000 ng/mL	+++	+++	Positive / Positive
6	Dope	Fentanyl (38.0%), 4-ANPP (9.6%), <i>para</i> -Fluorofentanyl (4.9%), Phenethyl-4-ANPP (trace), Lidocaine (0.6%)	0 ng/mL	---	---	Negative / Negative
7	Dope	Xylazine (43.1%), Fentanyl (8.1%), 4-ANPP (1.9%)	431,000 ng/mL	+++	+++	Positive / Positive
8	Dope	Xylazine (50.6%), Fentanyl (7.6%), 4-ANPP (0.7%)	506,000 ng/mL	+++	+++	Positive / Positive
9	Dope	Xylazine (39.3%), Fentanyl (18.7%), 4-ANPP (0.6%), Heroin (trace)	393,000 ng/mL	+++	+++	Positive / Positive
10	Dope	Xylazine (46.3%), Fentanyl (4.7%, 1p), 4-ANPP (5.9%), <i>para</i> -Fluorofentanyl (1.3%), Lidocaine (1.3%), Dimethylsulfone (0.2p)	463,000 ng/mL	+++	+++	Positive / Positive
11	Dope	Xylazine (42.8%), Fentanyl (10.3%), 4-ANPP (1.7%), Phenethyl-4-ANPP (trace)	428,000 ng/mL	+++	+++	Positive / Positive
12	Dope	Xylazine (40.5%), Fentanyl (12.9%, 1p), 4-ANPP (1.0%), Phenethyl-4-ANPP (0.1p)	405,000 ng/mL	+++	+++	Positive / Positive
13	Dope	Xylazine (24.9%), Fentanyl (19.7%), 4-ANPP (6.6%), <i>para</i> -Fluorofentanyl (1.3%)	249,000 ng/mL	+++	+++	Positive / Positive
14	Dope	Xylazine (11.5%), Fentanyl (3.2%, 1p), <i>para</i> -Fluorofentanyl (3.3%), <i>para</i> -Fluoro-4-ANPP (0.7p), <i>para</i> -Fluoro-Phenethyl-4-ANPP (0.1p), Caffeine (0.6p)	115,000 ng/mL	+++	+++	Positive / Positive
15	Dope	Xylazine (8.2%), Fentanyl (3.4%, 1p), <i>para</i> -Fluorofentanyl (3.5%), 4-ANPP (0.7%), <i>para</i> -Fluoro-4-ANPP (0.7p), <i>para</i> -Fluoro-Phenethyl-4-ANPP (0.1p), Caffeine (0.6p)	82,000 ng/mL	+++	+++	Positive / Positive
16	Dope	Fentanyl (12.8%), <i>para</i> -Fluorofentanyl (0.8%), 4-ANPP (1.8%), Phenethyl-4-ANPP (trace), <b>Lidocaine (1.3%)</b> , Dimethylsulfone (trace)	0 ng/mL	+-+	---	Negative / Negative*
17	Dope	Xylazine (45.2%), Fentanyl (22.4%, 1p), <i>para</i> -Fluorofentanyl (0.7%), 4-ANPP (5.3%), Phenethyl-4-ANPP (0.1p), Caffeine (0.1p)	452,000 ng/mL	+++	+++	Positive / Positive
18	Dope	Xylazine (55.5%), Fentanyl (1.9%, 1p), 4-ANPP (trace), <i>N</i> -Desethyl Isotonitazene (0.3p), Bromazolam (0.7p), Flubromazepam (0.1p)	555,000 ng/mL	+++	+++	Positive / Positive
19	Dope	Xylazine (36.5%), Fentanyl (4.6%, 1p), <i>para</i> -Fluorofentanyl (1.2%), 4-ANPP (1.3%), Phenethyl-4-ANPP (trace), Caffeine (0.4p)	365,000 ng/mL	+++	+++	Positive / Positive
20	Dope	Xylazine (44.8%), Fentanyl (6.2%, 1p), 4-ANPP (0.7%), Caffeine (0.3p), Lidocaine (0.9%)	448,000 ng/mL	+++	+++	Positive / Positive
21	Dope	Xylazine (7.5%), Fentanyl (3.3%, 1p), <i>para</i> -Fluorofentanyl (3.5%), 4-ANPP (0.4%), <i>para</i> -Fluoro-4-ANPP (0.2p), Dimethylsulfone (3.8p), Lidocaine (9.4%)	75,000 ng/mL	+++	+++	Positive / Positive
22	Cocaine	Cocaine (42.2%, 1p), <b>Lidocaine (10.0%)</b> , Dimethylsulfone (0.5p)	0 ng/mL	+++	---	Negative / Negative*
23	Cocaine	Cocaine (29.7%, 1p), Methamphetamine (11.1%), <b>Lidocaine (2.9%)</b> , Dimethylsulfone (0.9p)	0 ng/mL	+++	---	Negative / Negative*
24	Cocaine	Cocaine (88.2%), Methamphetamine (2.1%)	0 ng/mL	---	TNP	Negative / Negative
25	Cocaine	Xylazine (10.1%), Cocaine (25.8%, 1p), Fentanyl (3.9%), Lidocaine (5.3%), Dimethylsulfone (0.6p), Caffeine (trace)	101,000 ng/mL	+++	+++	Positive / Positive
26	Cocaine	Ketamine (1p), Caffeine (0.1p)	0 ng/mL	---	TNP	Negative / Negative
27	Cocaine	Cocaine (56.7%), Levamisole (11.0%)	0 ng/mL	---	TNP	Negative / Negative
28	Cocaine	Xylazine (6.7%), Cocaine (29.8%, 1p), Fentanyl (0.4%), Lidocaine (7.9%), Dimethylsulfone (0.5p)	67,000 ng/mL	+++	+++	Positive / Positive
29	Cocaine	Cocaine (29.8%, 1p), <b>Lidocaine (8.5%)</b> , Dimethylsulfone (0.3p), Phenacetin (0.1p)	0 ng/mL	+++	+++	Negative / Positive
30	Cocaine	Cocaine (6.5%, 1p), <b>Lidocaine (54.0%)</b> , Dimethylsulfone (0.7p)	0 ng/mL	+++	+++	Negative / Positive
31	Crack	Cocaine (94.8%)	0 ng/mL	---	TNP	Negative / Negative
32	MDMA	MDMA	0 ng/mL	---	TNP	Negative / Negative
33	Meth	Methamphetamine (92.6%)	0 ng/mL	---	---	Negative / Negative
34	Meth	Methamphetamine	0 ng/mL	---	---	Negative / Negative

Key: Test 1 = 5 mg in 5 mL; Test 2 = 5 mg in 30 mL; TNP = Test Not Performed; \* = Negative on Test 2 (Dilution)