Evaluation of Instanosis Xylazine Test Strips

DRUG CHECKING

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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 has been increasingly identified as an adulterant within the recreational drug supply. Originally formulated as a veterinary sedative, xylazine causes extended sedation and opioid-like CNS depressant effects within human. However, xylazine is alpha-2 adrenergic agonist and the opioid overdose antidote, naloxone, is an ineffective reversal agent. Routine exposure to xylazine has caused sever skin ulcerations and necrotizing tissue infections. Pharmacologically, xylazine and fentanyl have combined effects that result in eliciting a prolonged response or high. Increased co-positivity of stimulants has been reported which may be used to counteract the xylazine's sedating effect. From 2019 to 2022, xylazine reports more than doubled for 30 states and 43 states reported at least one xylazine-related overdose. During the same period fentanyl overdoses in which xylazine was detected increased 276% and were primarily concentrated in the Northeast (MD, CT, PA). Due to widespread detection of xylazine across the United States, lateral flow immunoassay test strips have been developed as a field-based drug checking tool for applications within law enforcement, point of care, and harm reduction sectors. Implementation of xylazine test strips to determine if a sample contains xylazine will likely be rapid given the widespread acceptance and use of 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 Instanosis. The XTS had a specification cut-off of 10 ng/mL. The lot number of the batch evaluated was X07241 Ref. XYL24.

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 replicates of five. 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 duplicate 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. Separate analysts performed XTS replicates and drug screening/quantitation.

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 1-5 mg micro scoop was used, depending on sample volume available, to aliquot the drug material into a test tube and 5 mL of tap water was added. A positive result was read if the Control (C) was present and Test (T) line was absent.

Results: The XTS cut-off was observed to be approximately 10 ng/mL. No false positives from various sources of water were discovered. No other drugs were found to exhibit false positives (or interferences) with the XTS. Thirty-two authentic drug materials were tested using XTS, including "dope", cocaine, "crack", methamphetamine, and MDMA samples. The XTS accurately identified all samples that contained xylazine (n=19) throughout the study. Two samples containing trace levels of xylazine also yield positive results, despite concentrations being below the cutoff, which may result from additional xylazine derivatives being in the samples that is not within the scope of analytical method.

Conclusion: The performance of the xylazine test strips was deemed acceptable for drug checking purposes, demonstrating high sensitivity (100%), specificity (100%), and precision (100%).

Future Studies: Future studies will be explored, including but not limited to continued testing of authentic samples within the evolving drug supply, testing of additional XTS batch lots, additional potentially interfering drugs, macro-dose interference studies to test higher concentrations of drugs relative to xylazine, and assessment of the test strips using mock and authentic biological specimens.

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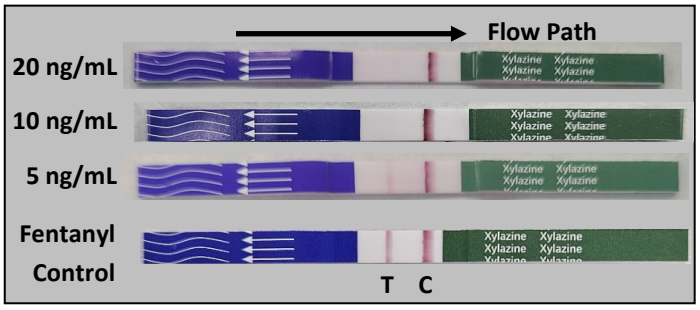
Negative Controls		
Samples	**Concentration**	**Results (n=5)**
Water (DI-1)	0 ng/mL	- - - - -
Water (DI-2)	0 ng/mL	- - - - -
Water (Tap-1)	0 ng/mL	- - - - -
Water (Tap-2)	0 ng/mL	- - - - -
Water (Tap-3)	0 ng/mL	- - - - -
Water (Bottled-1)	0 ng/mL	- - - - -
Water (Bottled-2)	0 ng/mL	- - - - -
Water (Bottled-3)	0 ng/mL	- - - - -

Xylazine Controls		
Drug	**Concentration**	**Results (n=5)**
Xylazine Standard	20 ng/mL	+++++
Xylazine Standard	10 ng/mL	+++++
Xylazine Standard	5 ng/mL	- - - - -



Interference Controls		
Drug	**Concentration**	**Results (n=5)**
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	- - - - -
Methamphetamine	10,000 ng/mL	- - - - -
Phenacetine	10,000 ng/mL	- - - - -
Procaine	10,000 ng/mL	- - - - -
Quinine	10,000 ng/mL	- - - - -
Theophylline	10,000 ng/mL	- - - - -
Fentanyl	10,000 ng/mL	- - - - -
BTMPS	10,000 ng/mL	- - - - -
Medetomidine	10,000 ng/mL	- - - - -
Metamizole	10,000 ng/mL	- - - - -

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AUTHENTIC DRUG MATERIALS				
#	**Type**	**Sample Contents (% purity)**	**Test: Results (n=2)**	**XTS Results: Expected vs. Observed**
1	Dope	4-ANPP, Despropionyl ortho-Chlorofentanyl, Fentanyl, Phenethyl 4-ANPP, Xylazine (3.9%)	+ +	Positive / Positive
2	Dope	Procaine, Xylazine (4.3%), Medetomidine, Bromazolam, Fentanyl, 4-ANPP, Quinine, Methamphetamine, 2-fluoro-2-oxo PCE, Tiletamine	+ +	Positive / Positive
3	Dope	Xylazine (9.5%), Quinine, Fentanyl, Caffeine, Heroin, para-fluorofentanyl, Procaine, 4-ANPP, Despropionyl para-fluorofentanyl, 6-MAM, Acetylcodeine	+ +	Positive / Positive
4	Dope	Acetaminophen, Lidocaine, Tramadol, Xylazine (< 1%), Medetomidine, 4-ANPP, para-Fluorofentanyl, Fentanyl, Phenethyl 4-ANPP	+ +	Positive / Positive
5	Dope	Caffeine, Fentanyl, Xylazine (4.8%) 4-ANPP, Phenethyl 4-ANPP, Quinine, Phenacetin	+ +	Positive / Positive
6	Dope	Fentanyl, Xylazine (32%), Procaine, 4-ANPP, Ethyl 4-ANPP, Acetyl Fentanyl , Lidocaine , Levamisole	+ +	Positive / Positive
7	Dope	Acetaminophen, Lidocaine, 4-ANPP, Ethyl 4-ANPP, Acetyl Fentanyl , Fentanyl, BTMPS, Phenethyl 4-ANPP	- -	Negative / Negative
8	Dope	Xylazine (40%), Procaine, Caffeine, Fentanyl, 2-fluoro-2-oxo PCE, Quinine, 4-ANPP, Bromazolam, Tiletamine, Ethyl 4-ANPP, Phenethyl 4-ANPP, para-Fluorofentanyl, Acetyl Fentanyl, Protonitazene, Cocaine	+ +	Positive / Positive
9	Dope	4-ANPP, Acetaminophen, Despropionyl para-fluorofentanyl, Fentanyl, Metamizole, para-Fluorofentanyl, Xylazine (1.2%)	+ +	Positive / Positive
10	Dope	Xylazine (37%), Procaine, Fentanyl, Caffeine, 2-fluoro-2-oxo PCE, Quinine, Bromazolam, 4-ANPP, Tiletamine, Ethyl 4-ANPP, Phenethyl 4-ANPP, Flubromazepam, Acetyl Fentanyl, Protonitazene, para-Fluorofentanyl, Cocaine	+ +	Positive / Positive
11	Dope	Phenacetin, Acetaminophen, Lidocaine, Metamizole, 4-ANPP, Despropionyl para-fluorofentanyl, para-Fluorofentanyl, Fentanyl, BTMPS, Xylazine (trace)	+ +	Positive / Positive
12	Dope	Procaine, Phenacetin, Fentanyl, Xylazine (2.9%), 4-ANPP, Heroin, Caffeine, Ethyl 4-ANPP, Phenethyl 4-ANPP, para-Fluorofentanyl	+ +	Positive / Positive
13	Dope	Procaine, Xylazine (25%), Caffeine, Heroin, Fentanyl, 6-MAM, Tetracaine, PCP, 2-Fluoro-2-oxo PCE, Quinine, 4-ANPP, Medetomidine, Acetylcodeine, Bromazolam	+ +	Positive / Positive
14	Dope	Xylazine (18%), Fentanyl, Quinine, 4-ANPP, Phenethyl 4-ANPP	+ +	Positive / Positive
15	Dope	Xylazine (46%), Caffeine, Procaine, Fentanyl, 2-fluoro-2-oxo PCE, Quinine, 4-ANPP, Bromazolam, Tiletamine, Ethyl 4-ANPP, Phenethyl 4-ANPP, Flubromazepam, Cocaine, Acetyl Fentanyl, para-Fluorofentanyl, Protonitazene	+ +	Positive / Positive
16	Dope	Acetaminophen, 4-ANPP, Despropionyl ortho-Fluorofentanyl, Fentanyl, para-Fluorofentanyl, para-Fluoro Phenethyl 4-ANPP	- -	Negative / Negative
17	Dope	Xylazine (23%), Fentanyl, Caffeine, para-Fluorofentanyl, 4-ANPP, Cocaine	+ +	Positive / Positive
18	Dope	Acetaminophen, Phenacetin, Caffeine, Xylazine (2.5%), Lidocaine, Tramadol, 4-ANPP, Cocaine, Acetyl Fentanyl, Quinine, Fentanyl, para-Fluorofentanyl, Phenethyl-4-ANPP	+ +	Positive / Positive
19	Dope	4-ANPP, Acetaminophen, Ethyl 4-ANPP, Fentanyl, Metamizole, para-Fluoro phenethyl 4-ANPP, para-Fluorofentanyl, Phenethyl 4-ANPP, Xylazine (3.9%)	+ +	Positive / Positive
20	Dope	Heroin, Fentanyl, 6-MAM, 4-ANPP, Acetylcodeine, Xylazine (1.3%), Caffeine, Phenethyl 4-ANPP, Phenacetin, Lidocaine	+ +	Positive / Positive
21	Dope	4-ANPP, Despropionyl para-fluorofentanyl, Para-fluorofentanyl, Fentanyl, para-Fluoro phenethyl 4-ANPP, Phenethyl 4-ANPP	- -	Negative / Negative
22	Dope	4-ANPP, Acetaminophen, Ethyl 4-ANPP, Fentanyl, Metamizole, Phenethyl 4-ANPP	- -	Negative / Negative
23	Dope	Fentanyl, Xylazine, (7.2%), 4-ANPP, Phenethyl 4-ANPP, Phenacetin	+ +	Positive / Positive
24	Dope	Heroin, Fentanyl, Xylazine (3.5%), Levamisole, 6-MAM, 2-fluoro-2-oxo PCE, 4-ANPP, Despropionyl para-fluorofentanyl, Acetylcodeine, para-Fluorofentanyl, Caffeine, Phenethyl 4-ANPP, Cocaine, para-Fluoro Phenethyl 4-ANPP, N-propionyl Norfentanyl	+ +	Positive / Positive
25	Dope	4-ANPP, Despropionyl para-fluorofentanyl, Acetyl Fentanyl, para-Fluorofentanyl, Fentanyl, para-Fluoro Phenethyl 4-ANPP, Phenethyl 4-ANPP	- -	Negative / Negative
26	MDMA	MDMA	- -	Negative / Negative
27	Meth	Methamphetamine	- -	Negative / Negative
28	DMT	DMT	- -	Negative / Negative
29	Cocaine	Phenacetin, Cocaine	- -	Negative / Negative
30	Dope	Acetaminophen, Metamizole, 4-ANPP, Fentanyl, Xylazine (trace)	+ +	Positive/ Positive
31	Benzo	Alprazolam	- -	Negative / Negative
32	Dope	Acetaminophen, Metamizole, 4-ANPP, Fentanyl	- -	Negative / Negative