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Characterization of Xylazine Test Strips for Use in Drug Checking

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PURPOSE

Xylazine is a veterinary drug commonly used as a sedative, analgesic, and muscle relaxant. Recently, xylazine is increasingly present as an adulterant in samples containing fentanyl and heroin. Reported dosages of 40 mg to 2,400 mg of xylazine are known to produce toxicity in humans. The consumption of xylazine can cause central nervous system and respiratory depression, along with other toxic effects such as hypotension, drowsiness, disorientation, staggering, or coma. In the worst-case scenario, this could potentially result in death.[1,2,3] According to the Drug Enforcement Administration, the lethal concentration of xylazine in humans is not established due to the overlapping concentration reported for both fatal and non-fatal cases.[3] Xylazine test strips are designed to provide a rapid presumptive result for the presence of xylazine. Public health analysts in the field can utilize these test strips without extensive training or sample preparation. Samples are dissolved in water and a few drops of the dissolved mixture are added to the test strip. Results are read between two to four minutes after sample introduction. The xylazine test strip produces one line in the control area in the presence of xylazine, while it produces one line in the control area and one line in test area for negative samples. Xylazine test strips not yet commercially available were obtained from Jiangnan University to be assessed for use on drug samples.

LIMIT OF DETECTION

The limit of detection (LOD) is the minimum concentration of xylazine in water that must be present to produce a visible positive result on the test strip. The manufacturer reported LOD was 300 ng/mL. The LOD study was conducted by analyzing solutions containing xylazine ranging from 250 ng/mL to 700 ng/mL in triplicate. The LOD was determined to be 650 ng/mL, as it was the lowest concentration that produced a positive response in all three replicates (see **Figure 1**).

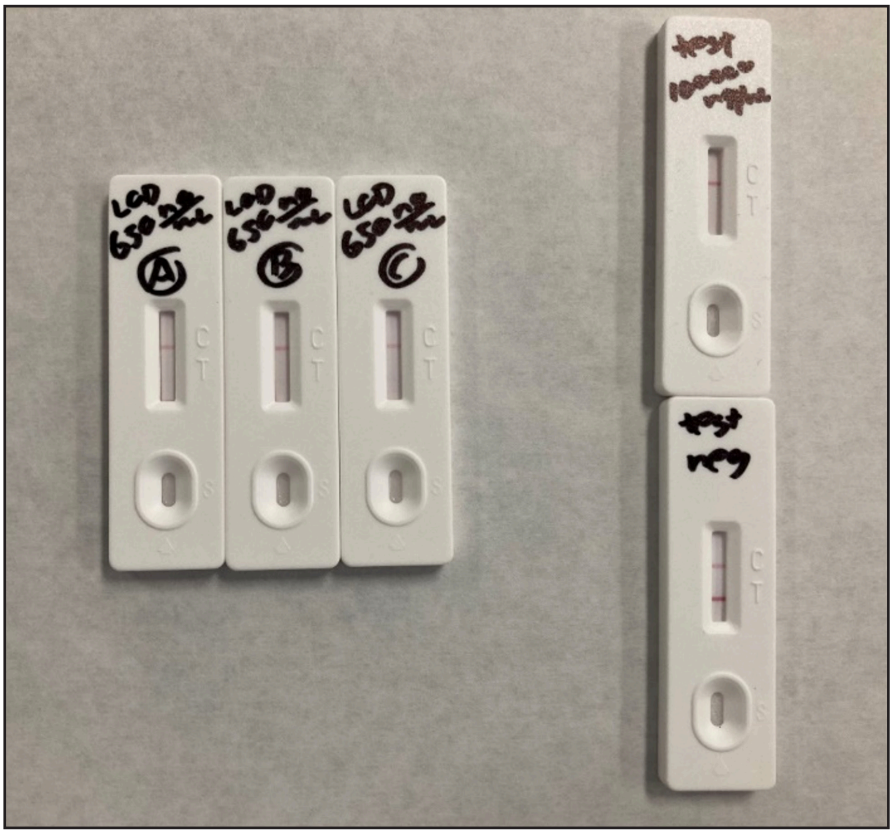

Figure 1-1: Limit of Detection (Left: 650 ng/mL of xylazine standard in triplicate; Top right: Positive control at 100,000 ng/mL; Bottom right: Negative control)

Figure 1-2: Concentration Below Limit of Detection (Left: 600 ng/mL of xylazine standard in triplicate; Top right: Positive control at 100,000 ng/mL; Bottom right: Negative control)

INTERFERENCE STUDIES

Heroin and fentanyl samples frequently contain xylazine as an adulterant. Many drug samples typically contain multiple controlled substances and adulterants. Other controlled substances, adulterants, and compounds commonly found in combination with xylazine were analyzed to determine if they would produce a false positive result on the xylazine test strips. The compounds assessed in this study included caffeine, diphenhydramine, cocaine, methamphetamine, MDMA, fentanyl, para-fluorofentanyl, phenacetin, lidocaine, levamisole, quinine, and heroin.

Initially, 1, 2, and 5 mg/mL concentrations of the potential interferents were evaluated. Caffeine, cocaine, methamphetamine, fentanyl, para-fluorofentanyl, phenacetin, lidocaine, and quinine produced a negative response at all three concentrations. A heroin standard was evaluated at 5 mg/mL and produced a negative response at this concentration, lower concentrations (1 and 2 mg/mL) were not tested as the highest concentration (5 mg/mL) produced a negative result. Diphenhydramine produced false positive results for concentrations ≥2 mg/mL in all three replicates. Levamisole produced false positive results for concentrations ≥1 mg/mL. Because a positive result was obtained for levamisole at a concentration of 1 mg/mL, additional testing was performed to determine the lowest concentration that would produce a false

positive. Concentrations of 0.1, 0.25, 0.5, and 0.75 mg/mL were evaluated. A positive result was observed for concentrations ≥0.5 mg/mL in all three replicates.

Some drugs and adulterants may be present in street samples at higher concentrations. Compounds that did not produce a positive result at 5 mg/mL, but may be present at elevated levels were analyzed at higher concentrations. Additional concentrations were based on those published by DanceSafe in their evaluation of fentanyl test strips.[4] Cocaine, methamphetamine, lidocaine, and phenacetin were tested at higher concentrations. Cocaine and phenacetin produced negative results at concentrations up to 25 mg/mL. At concentrations ≥15 mg/mL of methamphetamine, the result was recorded as inconclusive, as the test strips produced a very faint line in the test area that could conceivably be called negative depending on the observer. Lidocaine produced a false positive at concentrations ≥10 mg/mL. See **Table 1** for a summary of the results.

Concentration (mg/mL) / Compound	0.1	0.25	0.5	0.75	1	2	5	10	15	25
Caffeine	NT	NT	NT	NT	-	-	-	-	-	-
Diphenhydramine	NT	NT	NT	NT	-	+	+	NT	NT	NT
Cocaine	NT	NT	NT	NT	-	-	-	-	NT	-
Methamphetamine	NT	NT	NT	NT	-	-	-	-	*	*
MDMA	NT	NT	NT	NT	-	-	+	NT	NT	NT
Fentanyl	NT	NT	NT	NT	-	-	-	NT	NT	NT
Para-Fluorofentanyl	NT	NT	NT	NT	-	-	-	NT	NT	NT
Phenacetin	NT	NT	NT	NT	-	-	-	-	-	-
Lidocaine	NT	NT	NT	NT	-	-	-	+	+	+
Levamisole	-	-	+	+	+	+	+	+	+	NT
Quinine	NT	NT	NT	NT	-	-	-	NT	NT	NT
Heroin	NT	NT	NT	NT	NT	NT	-	NT	NT	NT

Table 1: Interference Study: "+" indicates a positive result, "-" indicates a negative result, "" indicates an inconclusive result, and "NT" indicates not tested.*

ANALYSIS OF KNOWN SAMPLES

Authentic samples submitted for drug checking were also assessed as part of the evaluation. The samples were powdered materials or residues. Samples were analyzed by GC/MS and LC-QTOF to identify drugs and adulterants present prior analysis using the test strips. A spatula-tip full of sample (approximately 0.2-

0.4 mg) was transferred into a 13x100 mm test tube. 1 mL of water was added, and the tube was vortexed to mix. For samples that did not contain a sufficient amount of powdered material, a cotton swab wet with water was used to swab the sample. The swab was then transferred into a 13x100 mm test tube and rinsed with 1 mL of water. The tube was vortexed to mix the sample solution. Three drops of sample solution were added to the test strip loading area, and the strip was allowed to develop for two to four minutes.
A total of twenty-five samples were analyzed. Twenty samples contained xylazine. All twenty samples containing xylazine produced positive responses on the xylazine test strips. Five samples did not contain xylazine. All five samples produced negative response on the xylazine test strips. Results from GC/MS and LC-QTOF, as well as xylazine test strips are shown in **Table 2** below.

Sample	**Identification on GC/MS and LC-QTOF**	**Xylazine Test Strip Response**
1	Fentanyl, Xylazine, 4-ANPP	Positive
2	Fentanyl, Xylazine, Para-Fluorofentanyl, 4-ANPP	Positive
3	Fentanyl, Xylazine, Lidocaine, 4-ANPP	Positive
4	Fentanyl, Xylazine, 4-ANPP	Positive
5	Fentanyl, Xylazine, Methamphetamine, 4-ANPP	Positive
6	Fentanyl, Xylazine	Positive
7	Fentanyl, Xylazine, Lidocaine, 4-ANPP	Positive
8	Fentanyl, Xylazine, 4-ANPP	Positive
9	Fentanyl, Xylazine, Para-Fluorofentanyl, 4-ANPP	Positive
10	Fentanyl, Xylazine, Caffeine, 4-ANPP, Noscapine, Phenethyl 4-ANPP, N-propionyl norfentanyl, Cocaine	Positive
11	Fentanyl, Xylazine, Caffeine, 4-ANPP, Phenethyl 4-ANPP, Noscapine, N-propionyl norfentanyl, Cocaine, Acetylfentanyl	Positive
12	Fentanyl, Xylazine, Caffeine, Para-Fluorofentanyl, 4-ANPP, Phenethyl 4-ANPP, Noscapine, N-propionyl norfentanyl, Cocaine, Acetylfentanyl	Positive
13	Methamphetamine	Negative
14	Fentanyl, Heroin, Xylazine, 4-ANPP, Acetylcodeine, Phenethyl 4-ANPP, 6-MAM, Noscapine	Positive
15	Fentanyl, Heroin, Xylazine, 6-MAM, 4-ANPP, Acetylcodeine, Phenethyl 4-ANPP, N-propionyl norfentanyl	Positive
16	Fentanyl, Heroin, Xylazine, Para-Fluorofentanyl, 6-MAM, 4-ANPP, Morphine, Acetylcodeine, Caffeine, Cocaine, Phenethyl 4-ANPP, Noscapine	Positive
17	Fentanyl, Heroin, Xylazine, Caffeine, Para-Fluorofentanyl, 6-MAM, 4-ANPP, Cocaine, Acetylcodeine, Phenethyl 4-ANPP	Positive

18	Fentanyl, Xylazine, Para-Fluorofentanyl, Caffeine, Cocaine, Para-fluoro phenethyl 4-ANPP, Despropionyl para-fluorofentanyl, Valeryl fentanyl, Phenethyl 4-ANPP	Positive
19	Fentanyl, Xylazine, Para-Fluorofentanyl, Cocaine, Para-fluoro phenethyl 4-ANPP, Despropionyl para-fluorofentanyl, Valeryl fentanyl, Phenethyl 4-ANPP	Positive
20	Fentanyl, Heroin, Xylazine, Para-Fluorofentanyl, Caffeine, Lidocaine, 6-MAM, 4-ANPP, Acetylcodeine, Para-fluoro phenethyl 4-ANPP, Papaverine, Noscapine	Positive
21	Cocaine, Phenacetin	Negative
22	Cocaine, Levamisole	Negative
23	MDMA	Negative
24	Fentanyl, Heroin, Xylazine, Para-Fluorofentanyl, Caffeine, Quinine, 6-MAM, 4-ANPP, Acetylcodeine, Para-fluoro phenethyl 4-ANPP, Phenethyl 4-ANPP	Positive
25	Fentanyl, Heroin, Para-Fluorofentanyl, Lidocaine, 4-ANPP, Phenethyl 4-ANPP	Negative

Table 2: Method Comparison

ROBUSTNESS

The instructions for the xylazine test strips indicated that 140 µL of sample should be added to the strip for testing. Analysts in the field might utilize plastic pasteur pipettes to deliver sample solution to xylazine test strips as plastic pasteur pipettes are cheap, disposable, and less fragile compared to laboratory pipettes that would be used to measure a precise volume. To determine the effect deviating from 140 µL would have on the results, various volumes were tested in triplicate. The volumes tested were 2, 3, and 4 drops of sample solution delivered by plastic pasture pipette. The drops of water were weighed to determine the average volume that was delivered in microliters. It was determined that 2 drops, 3 drops, and 4 drops delivered by plastic pasture pipette are equivalent to 66.2 µL, 115.5 µL, and 149.8 µL, respectively. Xylazine standard at 0.1 mg/mL, which was known to produce a positive result at 140 µL, was used for the analysis. The xylazine test strips produced a positive response for all three trials at the three different volumes. The analysis was also performed at the LOD (650 ng/mL). The xylazine test strips produced a positive response for all three trails at the three different volumes.

The instructions for the xylazine test strips indicated the results of the strip should be read and documented between two to four minutes after application of the sample. The positive samples from the volume robustness experiment outlined above were monitored and documented at 5 minutes, 10 minutes, 15 minutes, 30 minutes, and overnight at room temperature to observe any change in the result. No change was observed over the time timeframe monitored.

CONCLUSION

Xylazine test strips can reliably produce presumptive results of the presence of xylazine in drug samples. The test strips produced true positives for all the authentic drug samples containing xylazine and produced true negatives for all samples without xylazine. Sampling residue or a small amount of powdered material and diluting in 1 mL of water was sufficient sample preparation to produce solutions with a concentration above the LOD of the test strips.

Drug samples suspected or determined to contain high concentrations of diphenhydramine, lidocaine, levamisole, MDMA, or methamphetamine may produce a false positive for the presence of xylazine. False positives were not observed in drug samples containing these analytes during the assessment. Alternative methods may be required to determine the presence or absence of xylazine in drug samples containing interfering analytes.

The xylazine test strips are fit for the use in drug samples.

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