BRITISH COLUMBIA CENTRE ON SUBSTANCE USE
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PURPOSE OF THIS DOCUMENT

The purpose of this document is to provide people with what is known to date about xylazine test strips (XTS) as well as raise some of the considerations that have come from research into the validity and efficacy of these strips. The intention is to help inform the piloting of xylazine strips in drug checking services.

KEY TAKEAWAYS

- There have been 4 independent studies conducted to validate xylazine test strip performance and cross-reactivity
- XTS limit of detection is higher than advertised by both manufacturers (BTNX Inc., W.H.P.M Inc.) when used in the context of non-urine drug testing
- Lidocaine most commonly cross-reacts with XTS to give false positive results at as low as 10 ng/mL
- Due to cross-reactivity with cutting agents frequently used in stimulant drugs and stimulant drugs themselves, XTS are most useful in testing opiate samples for xylazine.
- XTS did not cross-react with other α_2-agonists such as medetomidine, which may lead to a false sense of security as other adulterants enter the drug supply
- For best results, proper dilution of samples is imperative; this means proper education and training on strip use should be prioritized
- Focus on XTS education and training is strongly recommended at this stage; No studies to date have followed XTS implementation in real-world settings.

STUDIES CITED IN THIS REPORT

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WHAT WE KNOW ABOUT XYLAZINE

The presence of xylazine, a veterinary tranquilizer, in the street drug supply has recently received significant media coverage across North America.[1,2] In the street drug supply, it is most commonly found as an adulterant in unregulated opioid samples (fentanyl, heroin) and there is currently limited information on the effect of combining opioids with xylazine and safe dosing for human consumption. The most commonly cited reasons for use among people who use drugs (PWUD) are that xylazine can produce a synergistic effect when combined with opioids, which can prolong the high.[3] However in most cases, xylazine is unexpected and not sought as a drug of preference.

XYLAZINE IN THE CANADIAN DRUG SUPPLY

Point of care drug checking technologies in British Columbia (BC) and Toronto have detected xylazine in the drug supply, primarily in samples expected to be opioids.[4]

- Among drug checking services provided by the BC Centre on Substance Use (BCCSU), xylazine is detected in low proportions of primarily opioid samples (Figure 1). Although detection of xylazine is low, there has been an increasing number of samples detected; 0.03% in 2019, 0.05% in 2020, 0.5% in 2021, 1.4% in 2022 and 2.0% in 2023 so far.*
- Comparatively, the Toronto drug checking project detected a higher prevalence of xylazine overall but has varied since 2020. In 2022, approximately 11% of expected fentanyl samples contained xylazine and this number has stayed consistent into 2023.**

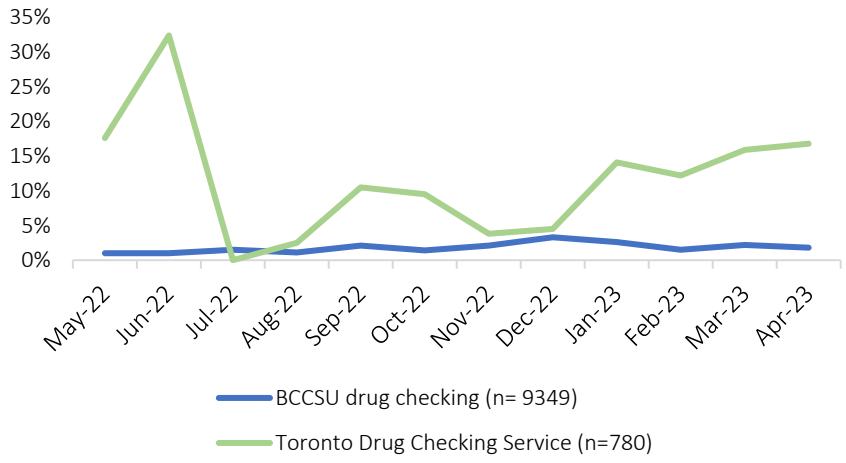

Figure 1. Proportion of expected opioid samples containing xylazine in samples submitted to drug checking services in British Columbia and Toronto, 2022-2023

* It is important to note that BCCSU drug checking uses Fourier-transform infrared spectroscopy (FTIR) which has a limit of detection of approximately 5%. Samples containing xylazine below this threshold would go undetected.
**The higher prevalence may be in part due to the lab-based technologies (i.e. gas and liquid chromatography) that are used, which are much more sensitive than FTIR.

XYLAZINE TEST STRIPS

Lateral flow Immunoassay testing strips have been proposed as a tool to identify xylazine as it enters the toxic drug supply. Similar to fentanyl and benzodiazepine testing strips, xylazine testing strips (XTS) confirm the presence or absence of the adulterant and may be used in tandem with other forms of drug checking to educate PWUD on the composition of their drugs [5–7]. Currently, two different producers of XTS exist in North America; BTNX Inc and WHPM Inc, both of whom advertise a limit of detection (LOD) of 1,000 ng/mL.

Proposed benefits of XTS as a harm reduction tool include the minimal training required to use them, their low LOD for xylazine, and the speed in which they provide results on the presence or absence of the adulterant [8]. However, few studies have tested XTS against real world drug samples, and no trials have followed the implementation of XTS in the field to assess their effectiveness as a harm reduction tool for PWUD.

SUMMARY OF XYLAZINE TEST STRIP PERFORMANCE – EVIDENCE TO DATE

To date, four independent researchers have studied XTS in the lab to better understand their usage as a harm reduction tool. Of the four studies, three used strips from the manufacturer BTNX Inc and one used strips manufactured by WHPM Inc. Below are the findings of each investigation summarized.

Study 1 *(Sisco et al., 2023)*[6]

In a study by Sisco et al., BTNX Inc. XTS were used to test lab standards and illicit drug residue samples to better understand the LOD, cross reactivity, reproducibility and sensitivity of available XTS.

Limit of Detection

A thirteen-point calibration curve (5000 ug/mL – 0.0 ug/mL Xylazine in solution) was used to determine the authentic LOD of the XTS. While the manufacturer stated a LOD of 1,000 ng/mL, this study found XTS to consistently detect xylazine in solutions of 2,500 ng/mL. Mixed XTS

results were seen when testing concentrations between 0.5 ug/mL and 1.0 ug/mL, and all samples less than 0.1 ug/mL were negative.
Overall, Sisco et al., states the LOD of these XTS to be above 2,000 ng/mL, with inconsistent results below 2,000 ng/mL and consistent results above 2,500 ng/mL.

Cross-reactivity

It is important to identify other compounds that may react with XTS to produce false positive (FP) results. Sisco et al., tested 77 compounds including:

- common illicit drugs (methamphetamine, MDMA, heroine, alprazolam, ketamine etc.)
- common cutting agents (Levamisole, lidocaine, caffeine etc.)
- α_2-agonists (Medetomidine, clonidine etc.)
- α_2-antagonists (Atipamezole, Yohimbine etc.), and
- compounds similar in structure to xylazine (Chlorpromazine, Imipramine etc.)

Each compound was diluted to 100 ug/mL and tested with two XTS. Of the 77 compounds, only lidocaine (a common cutting agent in stimulants and occasionally opiates) produced FP results at 100 ug/mL. Sisco et al., comment that while this is an optimistic finding, it also means that other α_2-agonists in the drug supply like medetomidine are going undetected, which may pose a future problem if concentrations of other α_2-agonists increase in the street drug supply.

Real world samples

To test authentic drug samples, 100 swabs were obtained from used drug paraphernalia in the USA and extracted before dilution in solution. Xylazine concentration was determined using GC-MS and DART-MS and then compared to XTS results. Of 100 samples:

- 66 samples contained xylazine above the LOD of GC-MS/DART-MS.
    - 65 of these xylazine positive samples gave positive XTS results
- Confirmatory testing detected no xylazine in 34 samples
    - 33 samples gave clear negative results
    - 1 sample gave a faint negative that could be mis-interpreted as positive

Specificity and Sensitivity

Calculated from authentic samples that had a xylazine concentration greater than 1,000 ng/mL AND samples found negative for xylazine. (n = 38 and n = 34 respectively).

Sensitivity	0.974
Specificity	1.00
Overall XTS accuracy	0.986

Sensitivity was calculated as the proportion of xylazine-containing samples in which XTS yielded positive results; **specificity** was calculated as the proportion of xylazine-absent samples in which XTS yielded negative results.

Recommendations

Sisco et al., points out the importance of properly preparing and diluting a drug sample prior to testing. In their study, XTS tested drug residue samples, but in a harm reduction setting, higher amounts of substance may be dissolved in water, leading to higher concentrations. Inaccurate results may come from samples that are improperly diluted, as extremely high concentrations of cross-reactive compounds have yet to be tested. Furthermore,

- Lot-to-lot variability should be considered; Re-evaluate LOD using different XTS lots from the same and different manufacturers.
- Test XTS against high concentrations of possible cross-reactive compounds
- Authentic drug samples donated by PWUD should be tested as opposed to authentic samples extracted from drug paraphernalia
- Studies of real-world applications are needed to explore the practicality of XTS use in the field.

Study 2 (Krotulski et al., 2023)[5]

This study was contracted by BTNX Inc. to examine accuracy and cross-reactivity of their XTS as used for individual drug checking purposes.

Limit of Detection

Lab standards and lab prepped authentic samples were used to test the LOD of BTNX Inx XTS. Xylazine standards from 100 ng/mL – 10,000 ng/mL were prepared in solution and tested while authentic samples were prepared using a 5 mg microscope and 5 mL of tap water (similar protocol currently used for FTS). If a suspicious result was found (such as a positive result in a stimulant samples), a second dilution was done with 30 mL of water. Concentration of all samples confirmed prior to XTS testing with GC-MS and LC-MS. XTS LOD was observed to be approximately 2,000 ng/mL or 2 ug/mL.

Cross-reactivity

- Of 11 compounds tested for interference, only lidocaine gave false positive results.
  - Consistently gave false positive results at 10,000 ng/mL or 10 ug/mL.
  - No false positive results when lidocaine was diluted to 1,000 ng/mL or 1 ug/mL.
  - Of n = 5 authentic samples with lidocaine, 3/5 gave negative results after second dilution with 30mL of water.
    - The remaining FP were cocaine samples with 8.5% and 54.0% lidocaine respectively.

Recommendations

- Test authentic samples that reflect the unregulated drug supply
- Test additional lots of XTS to account for lot-to-lot variability
- Test different interference drugs
- Assessment of XTS using mock and authentic urine specimens

Study 3 (WHPM Inc, 2023) W.H.P.M., Inc. Xylazine Test Strip - Substance Test Performance Characteristics White Paper May 2023[9]

The purpose of this study is to evaluate the performance of WHPM XTS available for distribution in North America for cross reactivity with relatively high concentrations of illicit substances (xylazine, fentanyl, MDMA and methamphetamine) and potentially cross-reacting cutting agents (diphenhydramine, phenacetin, lidocaine, levamisole, procaine and quinine) diluted in water. Substances were serially diluted from 10 ug/ml – 10 mg/ml and tests were performed in triplicate.

Cross-reactivity

- No cross reactivity with illicit substances was reported, indicating that these XTS are highly specific to xylazine when testing polysubstance compounds containing two or more of the illicit drugs
- Levamisole at 1 mg/mL gave false positive results, indicating cross-reactivity
- Lidocaine at 10 mg/mL produced a weak line which could be misinterpreted by the user

Recommendations

- The potential cross-reactivity may only be problematic if samples are improperly prepared and the substance is present in high concentrations
- Results are reassuring that XTS are accurate in identifying xylazine in solution, however this study did not examine XTS in real-world samples which is an important future direction

Study 4 (Data from new BTNX and WHPM xylazine test strips collected by Dr. M. Lieberman, U. Notre Dame, May 19 and 20, 2023)[10]

This data compared the performance of BTNX and WHPM xylazine test strips in samples containing illicit substance diluted in water. Researchers tested xylazine, lidocaine, levamisole, diphenhydramine, promethazine, ketamine, methamphetamine and fentanyl at varying concentrations.

Limit of detection

- WHPM XTS more consistently identified xylazine in solution at lower concentrations, closer to marketed LOD
- Both BTNX and WHPM test strips gave consistent positive results for xylazine samples with a concentration of 2000 ng/mL

Cross-reactivity

- BTNX XTS produced false positive results in lidocaine samples with a concentration of 10 mg/mL, promethazine at 1 and 10 mg/mL, ketamine at 10 mg/mL and methamphetamine at 2 mg/mL
- WHPM XTS produced false positive results in ketamine samples at 1 and 10 mg/mL
- Both BTNX and WHPM XTS produced false positive results in levamisole samples at concentrations of 1 and 10 mg/mL

Recommendations

- Many common cutting agents and substances produce false positives on XTS. Authors recommend that these strips should not be used to check for xylazine in drugs samples other than opioids.

GENERAL RECOMMENDATIONS FOR PILOT TESTING

1. Lidocaine, levamisole and other common cutting agents found in the drug supply have produced false positive results on xylazine test strips (XTS). Therefore, testing these compounds with XTS at varying concentrations can confirm cross-reactivity.
2. Evaluating XTS performance on samples containing other common adulterants in opioid samples, such as benzodiazepines and fentanyl analogues, is crucial to identify any cross-reactivity.
3. Pairing laboratory-based confirmatory testing with xylazine test strips for both real world xylazine-positive and xylazine-negative samples can be used to calculate the sensitivity, specificity, false negative rate and positive predictive value of the test strips.
4. Testing samples collected at point of care drug checking sites using FTIR and XTS can be used to determine the real-world implications of XTS use on samples from the unregulated drug supply.
5. Perform pilot testing in different regions as the unregulated drug supply is dynamic and not always consistent.
6. Perform pilot testing with various lots of xylazine test strips to account for lot-to-lot variability which can be high in immunoassay testing strips.

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