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Detection of xylazine by immunoassay test strips in community drug samples: Phase 2 report

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Land Acknowledgement

The British Columbia Centre on Substance Use would like to respectfully acknowledge that the land on which we work is the unceded territory of the Coast Salish Peoples, including the territories of the xʷməθkwəyəm (Musqueam), Sḵwxwú7mesh (Squamish), and səlílp lwətaɬ (Tsleil-Waututh) Nations.

We recognize that the ongoing criminalization, institutionalization, and discrimination experienced by people who use drugs disproportionately harms Indigenous peoples and that continuous efforts are needed to dismantle colonial systems of oppression. We are committed to the process of reconciliation with Indigenous peoples and recognize that it requires significant and ongoing changes to the health care system.

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Table of Contents

Background ***6***

Methods ***7***

Study design 7

Sensitivity and specificity of XTS 8

Results ***8***

Community samples 8

Diagnostic accuracy: Phase 2 samples 9

Diagnostic accuracy: Phase 1 and 2 samples 10

Discussion ***10***

Limitations 12

Conclusions ***12***

Considerations for service implementation ***13***

References ***15***

Appendices ***17***

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Background

The unregulated opioid supply in Canada is undergoing a new wave of adulteration with the introduction of veterinary tranquilizers—potent alpha-2 adrenergic receptor agonists that have emerged as a pressing public health concern. Xylazine, commonly referred to as "tranq," is frequently detected alongside fentanyl and its analogues, as well as benzodiazepines.[1] This polysubstance combination significantly elevates the risk of adverse health outcomes, including bradycardia, prolonged sedation, and overdose.[1] In a recent study, xylazine presence was also associated with a 126% increase in prevalence of prolonged sedation, and a 627% increased prevalence of seizure events.[2] Notably, xylazine is not responsive to naloxone, thereby complicating responses to opioid overdose events.[1,3]

The rising prevalence of xylazine has been well documented in Toronto, Ontario, where by April 2025, nearly half of all expected-fentanyl samples submitted for drug checking were found to contain xylazine.[4] However, it remains unclear whether British Columbia (BC) is experiencing similar trends, in part due to challenges in detecting xylazine at community drug checking services. Drug checking services in BC primarily rely on Fourier-transform infrared spectroscopy (FTIR), which can only detect xylazine when present in concentrations above the limit of detection [(LOD) approximately 5%].[5] At the end of 2023, 1.4% of expected-opioid samples submitted for drug checking in BC were found to contain xylazine by FTIR.[6] By March 2025, this number has only grown to 3%.[7] Given the demonstrated effectiveness of immunoassay test strips in detecting fentanyl and benzodiazepines at low concentrations,[8] xylazine test strips (XTS) have been proposed as a potential easy-to-use tool to improve xylazine detection at point of care.[9]

To date, there has been little investigation of the performance of XTS on real-world drug checking samples. To better understand their potential utility at point of care, we collaborated with our community partners and the Health Canada Drug Analysis Service to conduct a pilot study assessing the 1) diagnostic accuracy, 2) limit of detection, and 3) cross-reactivity of XTS manufactured by BTNX Inc.[10] In May 2024, we released a report presenting preliminary phase 1 findings on diagnostic accuracy, as well as results from our evaluation of the limit of detection and cross-reactivity of XTS while sample collection continued for phase 2.[11] This report presents results from our assessment of diagnostic accuracy on samples tested with XTS during phase 2, as well as overall sensitivity and specificity across samples collected from both phases. We also provide considerations for the implementation of XTS at community drug checking services at the end of this report.

Methods

Study design

Between February 1, 2024 and July 31, 2024, community drug checking partner sites in the Vancouver Coastal and Fraser Health regions of BC collected and tested drug samples with FTIR and XTS. All XTS were purchased from BTNX and originated from lot #DOAA2306042 and #DOA2309005. We note samples from phase 1 of this study had all been tested using XTS from lot #DOAA2306042.

As xylazine has been found predominantly in unregulated opioid samples, drug checking technicians prioritized using XTS on samples that service users expected be "down" (a mixture of unregulated opioids and diluents such as sugar and caffeine), and fentanyl, and as well as samples where the expected drug was unknown but were representative of unregulated opioids upon FTIR analysis. Technicians also performed XTS on samples that service users expressed concern about containing xylazine. These included stimulants (e.g., cocaine HCl, crack cocaine, and methamphetamine), benzodiazepines (e.g., alprazolam, unspecified benzodiazepines), and polysubstance samples (e.g., pre-mixed down and methamphetamine).

Technicians followed the XTS protocol provided on the BTNX product insert.[10] First, 5-10 mg of sample material was dissolved in 5 ml water. Technicians could scale this amount to 1-2 mg sample into 1 ml water to minimize the amount of sample needed from the service user to perform the test. For each sample, a XTS was dipped into the solution for 10-15 seconds, removed, and then left to develop for at least 1 minute before determining the result. As shown in **Figure 1**, the presence of a control band indicated the test had been performed properly. The presence of the test band (second band) indicated a negative result. The absence of the test band indicated a positive result. Technicians then logged the result in the BCCSU drug checking database.[12]

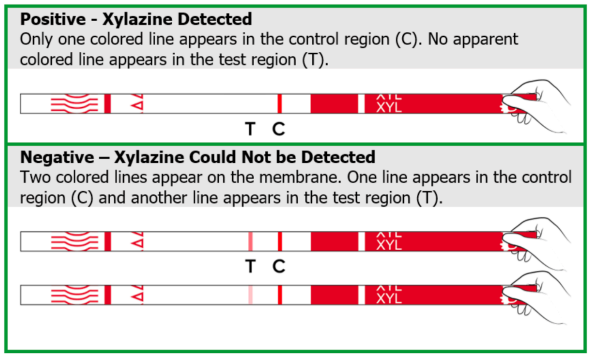

Figure 1. XTS result interpretation (BTNX product insert)

Sensitivity and specificity of XTS

A non-random, convenience subset of samples was saved for confirmatory analysis and sent to the Health Canada Drug Analysis Service (DAS).[13] The technologies used to confirm the presence or absence of xylazine included quantitative nuclear magnetic resonance (qNMR) and/or gas chromatography-mass spectrometry (GCMS). When possible, qNMR was used to determine xylazine concentrations. We then reported descriptive statistics, as well as measures of XTS sensitivity and specificity. Sensitivity was calculated as the proportion of true XTS-positive samples out of all the samples confirmed to contain xylazine by confirmatory analysis. Specificity was calculated as the proportion of true XTS-negative samples out of all the samples confirmed to not contain xylazine by confirmatory analysis. Next, we calculated overall sensitivity and specificity for samples included from phase 1 and 2 combined.

Results

Community samples

Between February 1, 2024 and July 31, 2024, community drug checking partner sites tested a total of 799 samples with XTS. Most samples tested with XTS were expected to be unregulated opioids: 723 samples were submitted as expected-down, and 31 as expected-fentanyl. The remaining samples tested with XTS included 25 unknown samples, 7 expected-stimulants, 9 expected-benzodiazepines, 3 polysubstance samples, and 1 expected-antihistamine sample. Of all the samples tested with XTS, 127 (15.9%) yielded positive results and 627 (84.1%) yielded negative results. Xylazine was detected by XTS in 116 (16.0%) expected-down samples, 10 (32.3%) expected-fentanyl samples, and in 1 (4.0%) sample where the expected drug was unknown. No expected-stimulant or expected-benzodiazepine samples tested positive for xylazine with XTS. Table 1 shows the types of drug samples tested, stratified by the proportion of samples that yielded xylazine-positive and negative results.

Of all the samples tested with XTS, 105 (13.1%) were sent to DAS for confirmatory analysis. Of that subset, 62 samples (59.0%) had tested positive for xylazine with XTS, and 43 (41.0%) samples had tested negative for xylazine with XTS. Appendix A provides a breakdown of the samples sent for confirmatory analysis by the expected drug type, stratified by XTS result.

Table 1. Community samples tested with xylazine test strips between February 1, 2024 and July 31, 2024 at drug checking sites in British Columbia (n=799)

Expected Substance	Total *N* = 799	Xylazine Test Strip Result, *n* (%)	
		Positive *n* = 127 (15.9)	Negative *n* = 627 (84.1)
Alprazolam	1	0 (0)	1 (100)
Benzodiazepine (Unknown)	8	0 (0)	8 (100)
Cocaine	3	0 (0)	3 (100)
Crack Cocaine	3	0 (0)	3 (100)
Down (Unknown Opioid)	723	116 (16.0)	607 (84.0)
Down and Methamphetamine	3	0 (0)	3 (100)
Fentanyl	31	10 (32.3)	21 (67.7)
Methamphetamine	1	0 (0)	1 (100)
Promethazine	1	0 (0)	1 (100)
Unknown	25	1 (4.0)	24 (96.0)

Diagnostic accuracy: Phase 2 samples

Of the 62 samples that yielded positive XTS results, confirmatory analysis found that xylazine was correctly detected in 41 (66.1%) samples. In 30 of these, xylazine concentration was measured by qNMR, and ranged from 0.4% and 11.8% by weight. Confirmatory analysis determined that XTS incorrectly detected xylazine (false positives) in the remaining 21 (33.9%) of XTS-positive samples. Of the samples that yielded false-positive results, 17 contained ortho-methylfentanyl, most in concentrations above 10% (wt/wt).

Of the 43 samples that produced XTS-negative results, confirmatory analysis determined the absence of xylazine in 42 (97.7%) samples. Notably, ortho-methylfentanyl was contained in 11 of these, with most in concentrations above 10% (wt/wt), including 2 in concentrations above 50% (wt/wt). Confirmatory analysis found 1 sample that produced a false-negative XTS result, however this sample contained xylazine at less than 0.1% concentration (wt/wt), which is below the XTS cut-off threshold of 1000 ng/ml. **Table 2** provides a breakdown of XTS-positive and negative samples sent to confirmatory analysis, stratified by whether or not xylazine was detected via confirmatory analysis (yes vs. no). From the samples tested in phase 2, we found the sensitivity and specificity of XTS to be 97.7%, and 66.7%, respectively.

Table 2. Phase 2 samples tested with XTS, stratified by confirmatory analysis result (n=105)

	Xylazine detected by confirmatory analysis (*n* = 105)		
XTS Result	Yes (%)	No (%)	Total
Positive	41 (66.1)	21 (33.9)	62
Negative	1 (2.3)	42 (97.7)	43

Diagnostic accuracy: Phase 1 and 2 samples

When combined with the results from phase 1, confirmatory analysis found that XTS correctly detected xylazine in 57 (70.4%) of the 81 samples that yielded positive XTS results, and produced false-positive results in 24 (29.6%) of samples. Of the XTS-negative samples, confirmatory analysis determined the absence of xylazine in 70 (98.6%) samples, and found false-negative XTS results in 1 (1.4%) sample. **Table 3** provides a breakdown of all XTS-positive and negative samples sent to confirmatory analysis for phases 1 and 2, stratified by detection of xylazine via confirmatory analysis (yes vs. no). Based on these results, we calculated the overall sensitivity of XTS to be 98.3%, and the specificity as 74.5% (**Table 4**). See **Appendix B** and **C** for a breakdown of phase 1 samples verified via confirmatory testing.

Table 3. Phase 1 and 2 samples tested with XTS, stratified by confirmatory analysis result (n=152)

XTS Result	Xylazine detected by confirmatory analysis (*n* = 152)		Total
	Yes (%)	No (%)	
Positive	57 (70.4)	24 (29.6)	81
Negative	1 (1.4)	70 (98.6)	71

Table 4. Summary of xylazine test strip diagnostic accuracy results

Sensitivity %	Specificity %
98.3	74.5

Discussion

This pilot study is one of the few to assess the efficacy of xylazine test strips on real-world samples, and contributes valuable insights into their practicality and limitations when used in the context of community drug checking services.

We found that overall sensitivity remained high across both phases of our study, observing only a small change from 100% sensitivity in phase 1 to 98.3% after including samples from phase 2. A

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reason for this change is that the sample which yielded a false-negative XTS result contained xylazine below the XTS cut-off threshold of 1000 ng/ml while following the prescribed protocol for dilution. We note that in our preliminary report, we had conflated the cut-off threshold with the LOD, and were since advised by the manufacturer that the cut-off indicates the concentration of xylazine that would correctly produce positive results 50% of the time, whereas the LOD is the concentration where samples would correctly test positive 95% of the time. To-date, several studies have established the LOD as 2000 ng/ml.[14-16]

We also observed a reduction in specificity after including samples from phase 2, which decreased from 90.3% in phase 1 to 74.5% overall. Notably, across both phases, many samples that produced false-positive results were found to contain ortho-methylfentanyl, a novel fentanyl analogue which had become more common in our setting throughout the study period. While in phase 1 we found only 3 instances of false-positives – all of which contained ortho-methylfentanyl – the larger sample size we obtained in phase 2 provided clearer insights. Of the false-positive samples in phase 2, 81% contained ortho-methylfentanyl, with the majority in concentrations high enough to be detectable by FTIR (>10% wt/wt). However, not all samples containing ortho-methylfentanyl produced false-positive results. Additionally, in the ad-hoc cross-reactivity evaluation of ortho-methylfentanyl we conducted in phase 1, we found that ortho-methylfentanyl did not truly cross-react with XTS but rather produced faint negative results. Thus, it is likely that faint negative results were misclassified, and in turn, affected our measurement of specificity.

These findings speak to the limitations of how XTS results are subjectively interpreted, and how misclassification can be influenced by a host of factors, including the lighting in the testing environment, or how long the test strips are left to develop. While these factors were not an issue for easily discernable negative results, faint negatives could have appeared positive to some technicians at first, especially if the sample contained higher concentrations of ortho-methylfentanyl or if the XTS has developed for an insufficient amount of time. Despite the following the product instructions of waiting at least 1 minute to the test strip to develop, it seems that XTS require longer development time to obtain reliable results. For example, Scott and colleagues[14] determined in their evaluation that the optimal development time of XTS was at least 2 minutes, and Leiberman[16] found that cross-reacting compounds produced clearer negative results after 5 minutes of development time.

We also note that we did not include an evaluation of XTS cross-reactivity with medetomidine in the second phase of our study, however we also did not have any evidence that medetomidine contributed to the false-positive results we observed. Of the 9 medetomidine-containing samples in phase 2 that did not contain xylazine, 7 correctly yielded negative XTS results. The remaining 2 samples that were associated with false-positive results also contained ortho-methylfentanyl. This aligns with Sisco and colleagues'[17] cross-reactivity evaluation of medetomidine in a previous study where no cross-reactivity was observed, and further supports our inference that the presence of ortho-methylfentanyl is linked with ambiguous XTS results.

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Limitations

As mentioned previously, an important limitation of conducting this pilot study in a real-world setting was the difficulty of controlling for all variability in XTS testing procedures and result interpretation. Although technicians followed the instructions provided on the XTS product insert,[10] procedures were not completely standardized. For example, BTNX recommends 5-10 mg of sample material in 5 ml of water (or 1-2 mg of sample if scaled), however, because technicians do not have access to highly precise weighing instruments, the amount of sample material would have varied and could have been over- or underestimated. Although not feasible during this pilot, controlling for variability in point of care XTS testing procedure and result interpretation may have produced different results in diagnostic accuracy.

Another limitation is that since this study was conducted, a second version of XTS has been developed by BTNX. It is unclear, however, how these XTS compare to the version we evaluated, especially in the presence of ortho-methylfentanyl. Since the release of the second version of XTS, BTNX has advised that they are in the process of examining cross-reactivity with ortho-methylfentanyl. Additionally, a recent evaluation of the new version of XTS have reported improvements, including increased sensitivity, and no cross-reactivity with lidocaine[9]. In contrast, previous studies, including our phase 1 report, found cross-reactivity of the first version of XTS with lidocaine[11,16].

Conclusions

As the prevalence of xylazine continues to rise in Canada, it is important to evaluate various methods that could aid in detection at point of care. Improved detection of xylazine in British Columbia has the potential to better characterize its presence, as well as inform harm reduction strategies among service users and broader public health responses. We found that BTNX XTS were highly sensitive, but limitations regarding specificity remain. This highlights the need for thorough training if XTS are to be used at point of care, as well as ongoing evaluation of potential cross-reactivity with novel compounds as they emerge to monitor changes in diagnostic performance over time. While XTS can be helpful to detect xylazine, it is important to remember that they cannot detect other tranquilizers and adulterants. In the context of an increasingly complex unregulated drug supply, this raises the question of how many test strips would be necessary and could realistically be implemented to support community drug checking services, with considerations such as cost in mind.

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Considerations for service implementation

Through this pilot, we determined that XTS can substantially enhance xylazine detection at point of care due to their high sensitivity, however, there are important considerations to take into account regarding their specificity in the context of a rapidly changing unregulated drug supply.

Samples

XTS appear to cause false-positive/faint negative results when a sample contains ortho-methylfentanyl, particularly in higher concentrations.

- Consider:
    - Only using on samples where ortho-methylfentanyl is not detectable by FTIR. If ortho-methylfentanyl is present in low concentrations, it is unlikely to react with the XTS
    - If deciding to use XTS on all opioid samples, when ortho-methylfentanyl is detectable by FTIR, use a lower concentration of sample to solution (2 mg in 2 ml solution) to minimize chance of yielding a faint negative that could be misclassified

Procedures

XTS appear to have a longer development time than other test strips to produce reliable results.

- Consider:
    - Waiting at least 2 minutes before interpreting the result
    - If the test strip appears positive at first, waiting at least 5 minutes before interpreting result
    - Plan workflow to ensure efficient testing with other methods, especially at high-volume sites (e.g., scaling sample amount used in order to test simultaneously with benzodiazepine test strip[18])

Training

Technicians must be trained on how to use XTS properly (procedure, workflow, explaining limitations)

- Consider:
    - Mandatory orientation to their usage
    - Making available a reference guide with testing protocol, and guidance on how to convey limitations to service users “e.g., because of the unpredictable nature of the drug supply, it is always possible that another substance is cross-reacting”

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Ongoing monitoring

It will be necessary to evaluate potential cross-reactivity with novel compounds as they emerge in the unregulated opioid supply to monitor changes in diagnostic performance over time via confirmatory testing.

- Consider:
    - Regularly spot-checking different types of samples tested with XTS per month to be verified by confirmatory testing, e.g., XTS+ sample with high concentration ortho-methylfentanyl, XTS+ samples with no xylazine detected by FTIR, XTS with faint negative

Cost

At present, XTS cost $275 CAD per box of 100. Usage of XTS may depending on funding capacity.

- Consider:
    - Only using XTS on opioid samples
    - Not using XTS on samples where xylazine is clearly detectable by FTIR

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Appendices

Appendix A. Phase 2 samples sent to confirmatory analysis, stratified by XTS result (n=105)

Expected Substance	Xylazine Test Strip Result		Total (%)
	Positive (%)	Negative (%)	
Down (Unknown Opioid)	59 (62.1)	36 (37.9)	95 (90.5)
Fentanyl	2 (50.0)	2 (50.0)	4 (3.8)
Cocaine	0 (0.0)	1 (100)	1 (1.0)
Unknown	1 (20.0)	4 (80.0)	5 (4.8)
Total	62 (59.0)	43 (41.0)	105 (100)

Appendix B. Phase 1 samples sent to confirmatory analysis stratified by XTS result (n=47) [a]

Expected Drug Type	Xylazine Test Strip Result		Total (%)
	Positive (%)	Negative (%)	
Down (Unknown Opioid)	15 (31.9)	16 (34.0)	31 (66.0)
Fentanyl	2 (4.3)	9 (19.2)	11 (23.4)
Methamphetamine	0 (0.0)	1 (2.1)	1 (2.1)
Unknown	2 (4.3)	2 (4.3)	4 (8.5)
Total	19 (40.4)	28 (59.6)	47 (100)

[a]Results reported previously in phase 1 findings

Appendix C. Phase 1 samples tested with XTS stratified by confirmatory analysis results (n=47)[a]

XTS Result	Xylazine detected by confirmatory analysis (*n* = 47)		Total
	Yes (%)	No (%)	
Positive	16 (84.2)	3 (15.8)	19
Negative	0 (0.0)	28 (100.0)	28

[a]Results reported previously in phase 1 findings

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