BRITISH COLUMBIA CENTRE ON SUBSTANCE USE

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Report

The effect of water temperature and agitation on benzodiazepine test strip detection

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Land Acknowledgement

The BC Centre on Substance Use would like to respectfully acknowledge that the land on which we work is the unceded ancestral homelands of the xʷməθkʷəy̓əm (Musqueam), Sḵwx̱wú7mesh (Squamish), and səlilwətaɬ (Tsleil-Waututh) Nations.

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For more information about drug checking services in the Interior Health region, visit https://drugchecking.ca/.

Information about HaRT, including services, locations and hours, can be found at https://hart.ok.ubc.ca/.

Learn more about drug checking services at: www.drugcheckingbc.ca

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Purpose of the Report

This report examines data collected from Interior Health and the Harm Reduction Team (HaRT) from the University of British Columbia Okanagan (UBCO) as part of a quality improvement initiative to determine the effect of solution agitation (i.e., shaking or vortexing) and water temperature on the detection of benzodiazepines, using benzodiazepine test strips. The purpose of this report is to provide insight into the detection of benzodiazepines by benzodiazepine test strips under two conditions at community drug checking services that use the test strips.

Interior Health and HaRT were interested in pursuing this experiment based on the rapid increase in detection of unexpected benzodiazepines in the unregulated drug supply in early 2021 leading to high demand for take-home benzodiazepine test strip availability.

List of Acronyms and Other Frequently Used Terms

BC: British Columbia

DCBC: The BC Centre on Substance Use drug checking database that hosts drug checking data collected across British Columbia

HaRT: Harm Reduction Team from the University of British Columbia Okanagan

FTIR: Fourier-transform Infrared spectrometer

OPS: Overdose Prevention Site

PS-MS: Paper-spray Mass Spectroscopy

SCS: Supervised Consumption Site

SERS: Surface-enhanced Raman Spectrometer

UBCO: University of British Columbia Okanagan campus

Unregulated Opioids: Term used in the report to refer to substances expected to contain opioids, including fentanyl, heroin, and/or colloquial "down"

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Summary of Key Findings

- Benzodiazepine test strips require additional procedural steps to maximize their detection compared to fentanyl test strips. Benzodiazepine test strips may require agitating the sample in a testing container to assist in the dissolution of the sample prior to testing.
- The amount of time the solution was agitated for (5 vs. 30 seconds) did have a significant effect on benzodiazepine test strip detection of benzodiazepines.
- Water temperature (room temperature vs hot water) did not have a significant effect on benzodiazepine test strip detection of benzodiazepines.

Background

Drug checking has evolved over the years in response to the unregulated drug supply, from reagent testing in music festival and rave settings, to test strips and spectrometers in community settings to combat the toxic drug crisis.[1,2] Various locations, such as supervised consumption sites (SCS), overdose prevention sites (OPS), and community centres started offering drug checking services as a community harm reduction tool after the public health emergency was declared in British Columbia (BC) in April 2016. The majority of drug checking services in BC use fentanyl test strips in conjunction with a Fourier-transform infrared (FTIR) spectrometer. The two technologies used together have been found to provide results with high sensitivity and specificity in a community setting.[3] The FTIR spectrometer is limited in the number of substances that can be determined from a drug checking sample, and the detection limit for compounds is 5-10%.[3] This means that compounds present in concentrations less than 5% may not be detected by the FTIR spectrometer. In contrast, the immunoassay strips have a higher sensitivity, but they are limited in their ability to detect fentanyl and benzodiazepine analogues and only give binary results.[3]

Benzodiazepines have become increasingly present in the unregulated opioid supply across BC, leading to benzodiazepine test strips being implemented at community drug checking sites. As of December 2022, benzodiazepines were present in 51.1% of unregulated opioid samples submitted for drug checking during that month, with etizolam being the most frequently detected compound within the benzodiazepines category in 2022.[4] Although etizolam is often included in the benzodiazepine category, it is technically a thienodiazepine which is structurally different from traditional benzodiazepines. Despite this, past research has shown that etizolam can be detected with benzodiazepine test strips, but at a reduced accuracy compared to traditional benzodiazepines such as alprazolam.[5]

We wanted to evaluate the detection of benzodiazepines with benzodiazepine test strips further by examining if the duration of agitation of the solution and water temperature has an impact on the dissolution of benzodiazepines and consequently, the accurate detection of the compound in community drug checking settings.

Methods

BTNX benzodiazepine test strips were used to test samples from HaRT's drug checking service between May 28, 2021 through February 15, 2022. A total of 202 samples were tested with both the FTIR spectrometer and benzodiazepine test strips, as confirmatory analysis was not available for HaRT. To assess both water temperature and solution agitation time on the detection of benzodiazepines, we evaluated four treatments:

1. Room temperature water (21°C) and solution agitation for 5 seconds
2. Room temperature water and solution agitation for 30 seconds
3. Hot water (55°C) and solution agitation for 5 seconds
4. Hot water (55°C) water and solution agitation for 30 seconds

All of the samples were tested with the 5- and 30-second treatments. Specifically, every sample was checked with a benzodiazepine test strip after agitating for five seconds and again after agitating for an additional 25 seconds. Samples were randomly assigned to hot or room temperature water using a coin toss, where Heads was assigned as the room temperature water treatment and Tails was assigned as the hot water treatment. The results of the test strip for each treatment was then logged on the data collection form. See **Figure 1** for a visual overview of the experimental design.

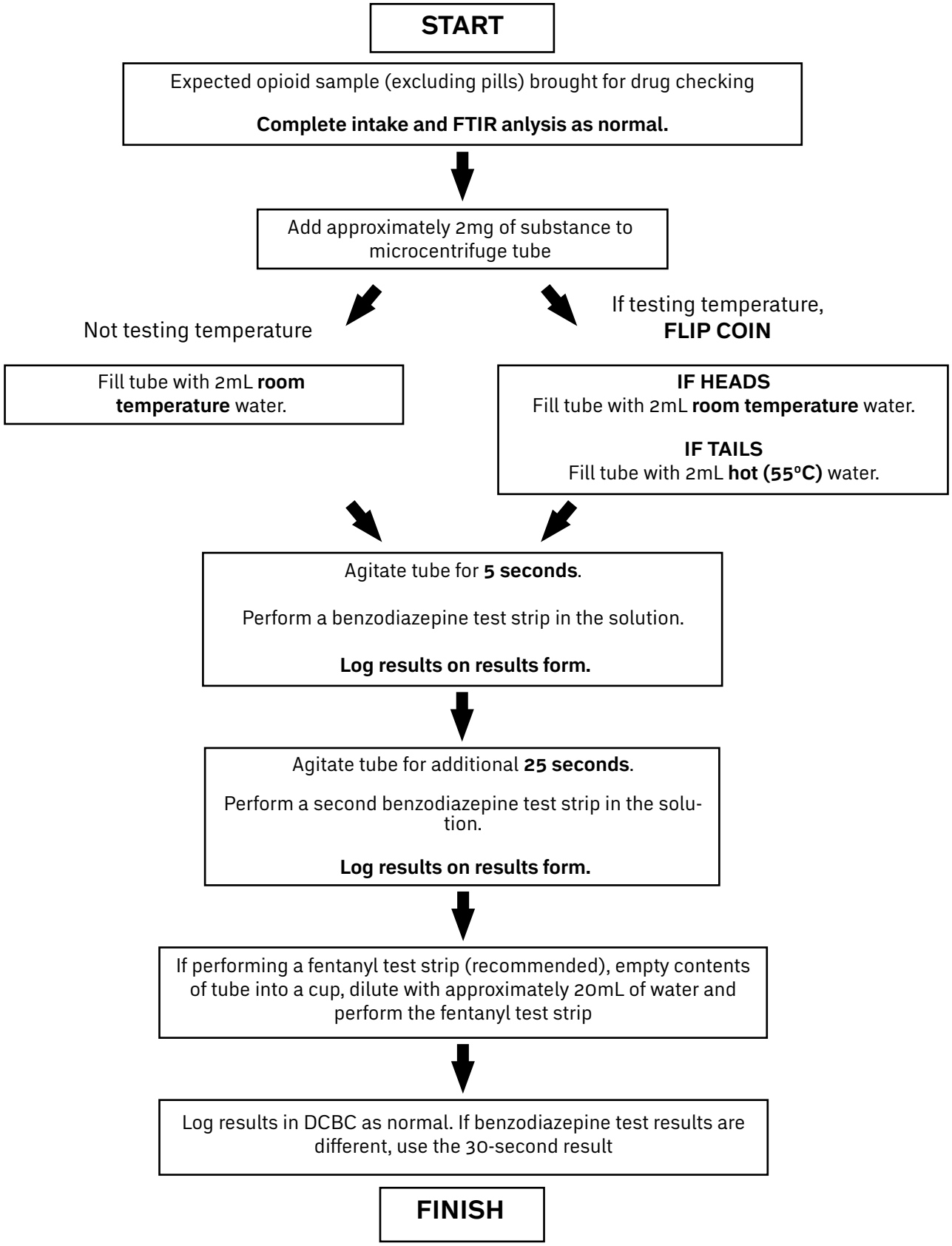

Figure 1. *Flow chart representation of the experimental design*

The data were analyzed using a Pearson's chi-square test, or Fischer's exact test if assumptions were not met, to compare the sensitivity values between the 5- and 30-second treatments. McNemar's test was used to determine the statistical significance of agitation time on the test strip sensitivity with room temperature water at 5 seconds and 30 seconds of agitation (treatment pair 1-2) and hot water at 5 seconds and 30 seconds of agitation (treatment pair 3-4).

Results

Of the 202 samples that were collected by HaRT, 188 were included in the present analysis as 14 (6.9%) had missing data. Of the included samples, 102 (54.3%) samples were assigned to room temperature water and 86 (45.7%) were assigned to hot water.

Solution Agitation Time

First, we examined whether agitation time impacts benzodiazepine test strip results, regardless of water temperature. After 5 seconds of agitation time, 73 samples (38.8%) tested positive and 115 samples (61.2%) tested negative. After 30 seconds, 85 (45.2%) samples tested positive and 103 (54.8%) tested negative. Using Fisher's exact test, we found a statistically significant difference in benzodiazepine detection at 5 and 30 seconds of solution agitation ($p <= 0.01$).

Next, we examined whether agitation time Impacts benzodiazepine strip results among samples assigned to room temperature water. Of the 102 samples assigned to room temperature water, 35 (34.3%) tested positive and 67 (65.7%) tested negative after 5 seconds of agitation time. After 30 seconds, 44 (43.1%) samples tested positive and 58 (56.9%) tested negative. Using McNemar's test, we found a statistically significant difference in test strip detection of benzodiazepines at 5 and 30 seconds of solution agitation ($p = 0.02$).

Finally, we examined whether agitation time impacts benzodiazepine strip results among samples assigned to hot water. Of the 86 samples assigned to

hot water, 38 (44.2%) tested positive and 48 (55.8%) tested negative after 5 seconds of agitation time. After 30 seconds, 41 (47.7%) samples tested positive and 45 (52.3%) tested negative. Using McNemar's test, we failed to find a statistically significant difference in test strip detection of benzodiazepines at 5 and 30 seconds of solution agitation ($p = 0.45$)

Water Temperature

We then explored if water temperature impacts benzodiazepine test strip results. Of the 102 samples assigned to room temperature water, 44 (43.1%) tested positive and 58 (56.9%) tested negative using test strips. Of the 86 samples assigned to hot water, 41 (47.7%) samples tested positive and 45 (52.3%) tested negative. Using a chi-square test, we failed to find a statistically significant difference in test strip detection of benzodiazepines in warm water ($p = 0.63$).

Limitations

This study has several limitations. First, data were collected from 2mg drug samples submitted by individuals to HaRT. Because the drug supply is unregulated, the composition and concentration of each sample can vary, which means the amount of benzodiazepine in a given sample cannot be controlled for in the experimental design. The sample variability was accounted for by collecting a sufficient sample size of greater than 200 samples in order to sufficiently determine the effect of agitation and water temperature on the test strip sensitivity.

Second, we do not actually know if benzodiazepines are truly present in the sample, as we were relying on the strip alone which we considered a presumptive test result. Benzodiazepine test strips have been shown to have less than 100% sensitivity and specificity, which means that benzodiazepine presence cannot be determined with complete certainty without confirmatory testing results.[3] This creates limitations in the conclusions that can be drawn from the results, as there is a possibility of benzodiazepine test strips providing a false positive result.

Conclusion

The data provide valuable insight on how solution preparation impacts benzodiazepine detection in community drug checking services when using benzodiazepine test strips. The results show that solution agitation time did have a significant effect on the detection of benzodiazepines with benzodiazepine test strips, but water temperature did not. Based on the findings, implementing 30-seconds of solution agitation to the drug checking procedure may help to reduce the occurrence of test strips reading false negatives on substances containing benzodiazepines.

The results of this report confirm that the benzodiazepine test strips do not always accurately detect benzodiazepines, in particular etizolam which was prevelant at the time of this study, despite the added measures of agitation and hot water to aid in the dissolution of the benzodiazepines. In addition, with the added steps required to prepare the sample, it does not make for a simple process for individuals using the benzodiazepine test strips at home to use with consistency. The concern from this finding is that there could be an increased risk of overdose for individuals using the test strips at home, as they may receive a false negative. At this time in BC, no regional health authorities have approved the use of benzodiazepine test strips for take-home use.

While the FTIR spectrometer and immunoassay strips work well in conjunction to provide drug checking results, they are still limited in their detection of benzodiazepines.[3] Quality improvement initiatives utilizing gold standard confirmatory analysis would be needed to further validate benzodiazepine test strips. With the emergence of new drug checking technologies, future research could be conducted to determine the detection of benzodiazepines using other technologies. Additionally, other factors could be evaluated for their effect on benzodiazepine detection with benzodiazepine test strips; for example, the concentration of the solution and the solute used. Drug checking technologies are evolving and being assessed for their use in community settings and are promising in their potential to improve the sensitivity and specificity of drug checking results in a community setting.[6]

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