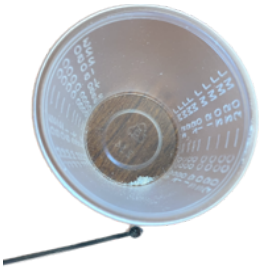
MADDS Test Strip Quick Reference Guide Updated April 2025

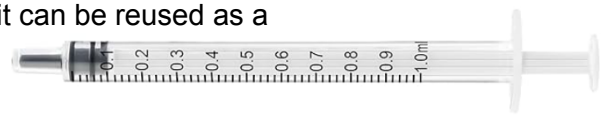
- Hold test strips from the solid end and dip the other side with the wavy lines into the water. Don't submerge past the solid line! After a few seconds, once the water appears to be moving upward via capillary action, remove the test strip and lay on a flat surface.
- Look closely and in direct bright light. Sometimes the second line on a test strip is very faint but should still be interpreted as a negative result. Faint lines could indicate a cross-reactive substance is present (see final page), for example, high levels of caffeine (as indicated on the FTIR) may cause a faint second line. Make note of this in the notes section on StreetCheck.
- Use sample cups with mL markings.
- Multiple test strips can be used in the same sample cup (and at the same time) if the recommended dilution is the same.



- If testing a sample with multiple strips (xylazine, fentanyl, and/or benzo), begin with the test strip that is the least sensitive (which requires the most concentrated solution). Adding water to decrease the concentration for more sensitive strips is the most efficient use of the sample provided by the participant.
- For the list of sensitivities of the strips and recommended dilutions please see the 1 page chart overview.
- For information on false positives and instructions around further dilutions please see the overview on Sensitivities, Concentrations, False Positives, and Cross-Reacting Substances
- Cooker/cotton/pipe samples have already been diluted and don't require as much water when creating a solution to test. Diluting cooker/cotton/pipe samples too much can result in false negative results.
- When possible, use microscoops to scoop your sample. For the orange scoops, the large end is ~10 mg, the small end is ~5 mg depending on the density of the powder. Black microscoops and the microscoops that come in the test strip packaging are ~10 mg.

- Use one microscoop per sample and dispose after use, or clean the microscoop very well with isopropyl alcohol in between samples to prevent cross-contamination.

- Tap water can raise the limit of detection of test strips making it harder for test strips to provide a true positive result. Utilize purified water ampules or consider increasing the concentration of the recommended dilution (use less water or add more sample).
- Use a 1 mL syringe to transfer water to the medicine cup if only 1 mL of water is needed. Do not contaminate the 1 mL syringe so it can be reused as a water transfer syringe in future testing.



Please be advised that test strips and the antibodies in the test strips may undergo modifications without prior notice and are not subject to federal oversight or regulation. This document may become outdated at any point and will be updated regularly to stay accurate and compliant.

If you are interested in sending used test strips to Marya at Notre Dame for secondary laboratory testing or if you have any questions please contact the MADDS team:maddsbrandeis@gmail.com

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1. BTNX Fentanyl Test Strips (FTS)

If a sample is a chunky **powder/crystal**:

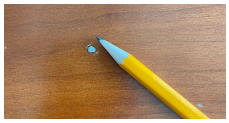
- Crush and mix the entire sample into a fine powder. Add a small amount of the sample to a plastic cup (approximately 5 mg or one microscoop if available). Add 5 mL of water to the cup. Swirl the solution. Test.

If a sample is a whole or partial **pill**:

- Crush and mix the pill into a fine powder. Add a small amount of the sample to a plastic cup (approximately 5 mg or one microscoop if available). Add 5 mL of water to the cup. Swirl the solution. Test.

If the sample is a **residue** in a cooker/cotton.

- Scrape the cooker bottom or roll the cotton between your gloved fingers to loosen about 1 mg of sample (see images below for reference) - remove the residue from the cooker and place into a cup. Add 1 mL* of water. Gently tilt the cup and swirl the solution. Test.



If a sample is a **residue** and not in a cooker (e.g. baggie with very little sample):

- Add approximately 1 mg of sample to a cup. Add 1 mL* of water to the cup. Gently tilt the cup and swirl the solution. Test.

*1 mL of water - use a 1 mL syringe - Covers about ⅔ of the bottom of the cooker when flat (see image below)

Add water directly to the cooker/baggie with residue as a last resort. This method may interfere with further testing (i.e. FTIR, GC/MS, LC/QToF/MS, etc.). Consider sending the used test strip(s) to Marya at Notre Dame for secondary laboratory testing.

BTNX FTS can positively detect the following fentanyl analogues at their respective cutoffs (ng/mL): Carfentanil (5,000), Butyryl Fentanyl (700), para-FluoroFentanyl (200), Acetyl Fentanyl (150), Fentanyl (200), Furanyl Fentanyl (500), Valeryl Fentanyl (700), Ocfentanil (250), 3-Methyl Fentanyl (500), Remifentanil (70,000), Sufentanil (100,000).

For a list of non cross-reacting substances (per BTNX) please check out their product insert linked here.

2. BTNX Xylazine Test Strips (XTS)

For testing **suspected opioid** samples for xylazine:

1. Crush and mix entire sample into a fine powder
2. Add 5 mg or 1 microscoop of sample to a plastic medicine cup. If 10 mg are available, use 10 mg.
3. Add 1 mL of water to the cup
4. Swirl the solution until dissolved
5. Test with BTNX XTS and interpret results.

For testing **suspected non-opioid** (cocaine/crack/meth/MDMA) samples for xylazine:

If FTS (at 1:1 ratio) is positive or an adverse experience suggests presence of xylazine, test sample with XTS. Create a concentrated solution that is 10-15 mg of sample per 1 mL of water for BTNX XTS. Do not use this solution to test with FTS. Be cautious that a high of a concentration like this could cause a false positive on XTS, especially if lidocaine is present.

Remember: There is always the risk for false results and a negative test strip result is not always reliable due to the high cut off. Xylazine may still be present even with a negative result. If possible, analyze the sample on FTIR and/or send the sample for secondary laboratory testing.

The latest version of XTS (2.0) released by BTNX has shown no cross-reactivity with Lidocaine. The sensitivity cut off for XTS is still 1000 ng/mL. It is important to keep track of LOT numbers of test strips by adding photos of the packaging to StreetCheck.

For more information and a complete list of non cross-reacting compounds, please see the BTNX product insert linked here.

If you're not certain if you have XTS 1.0 or 2.0, use FTIR to look for lidocaine if you suspect a false positive.

3. BTNX Benzo Test Strips (BTS)

If a sample is a whole or partial **pill/tablet** or **suspected Benzo**:

Crush and mix the pill/tablet into a fine powder. Add a small amount of sample (approximately 5 mg or one microscoop if available) to 2 mL of water. Be sure to **mix thoroughly, shake vigorously in a microcentrifuge tube, or use warm water** to dissolve the sample. Test with a benzo test strip.

What types of samples to test?

- Any pill or pill remnant samples
- Any sample suspected to have benzo in it as shared by the participant or identified by FTIR

BTNX Test Strip Detectable Benzos (300 ng/mL):

- Oxazepam (300), **Alprazolam (125)**, Bromazepam (625), Chlordiazepoxide (2500), Clobazam (63), Clonazepam (2500), Clorazepate (3300), Desalkylflurazepam (250), **Diazepam (250),** Estazolam (5000), Flunitrazepam (375), Flurazepam (>10,000), Lorazepam (1250), Lormetazepam (1250), Medazepam (>100,000), Midazolam (>100,000), Nitrazepam (25,000), NorChlordiazepoxide (250), Nordiazepam (500), Prazepam (>100,000), Temazepam (63), Triazolam (5000).

For more information look here:

- Benzodiazepines, Etizolam and the Test Strips, Substance Drug Checking
- Detection of etizolam, flualprazolam, and flubromazolam by benzodiazepine-specific lateral flow immunoassay test strips, BCCSU
- BTNX BTS Product Insert

4. BTNX Nitazene Test Strips (NTS)

Crush and mix the entire sample into a fine powder. Add 10 mg or two microscoops of sample to a plastic medicine cup. Add 1 mL of water to the cup. Swirl the solution. Test.

When deciding whether or not to use a Nitazine test strip, consider the following:

- Do you have sufficient sample (10 mg) for testing?
- Is the sample a suspected opioid?
- Are nitazenes potentially visible on FTIR? Is FTS Negative or FTIR shows <5% Fentanyl but the sample was reported to feel stronger than normal?
- Was the sample sold as a nitazene?
- Is the sample likely from the same batch/supplier/neighborhood as a recently confirmed nitazene result?
- Were there reports of pain/burning at injection site that travels along the vein or is associated with an adverse health event (e.g. OD)?

If more than one of these bullets are true for the sample, use a nitazene test strip.

Nitazene	Concentration (ng/mL)
Isotonitazene	2000
Protonitazene	3000
N-Pyrrolidono Etonitazene	2000

This table was adapted from BTNX Harm Reduction Website (https://www.btnx.com/product/nitazene-test-strips-ntz-18s26).

Desnitazenes are not detectable with BTNX NTS. Please see Nitazene test strips: a laboratory evaluation by Vrieze et al. (2024) in BMC's Harm Reduction Journal for a list of nitazene analogues screened and the cut offs where they were or weren't detectable.

BTNX reports NTS will not cross-react with common cutting agents (e.g. caffeine, acetaminophen, diphenhydramine), but this has not yet been evaluated in real-world samples. Please refer to the BTNX NTS Product Insert for more information.

5. BTNX Medetomidine Test Strips (MTS)

Crush and mix the entire sample into a fine powder. Add 10 mg or two microscoops of sample to a plastic medicine cup. Add 1 mL of water to the cup. Swirl the solution. Test.

When deciding when to use a medetomidine test strip, consider the following:

- Do you have sufficient sample (10mg) for testing?
- Is the sample a suspected opioid?
- Is the sample likely from the same batch/supplier/neighborhood as a recently confirmed medetomidine result?
- Were there reports of hallucinations? Were there reports of heavy sedation but you cannot identify xylazine on XTS or FTIR?

If one or more of these are true for your sample, please test with an MTS. Medetomidine can be difficult to identify on FTIR because it has overlapping peaks with many other common active cuts (e.g. xylazine) and is typically seen in low concentrations.

BTNX reported Levamisole has cross-reacted with MTS at 5 mg/mL but not at 2mg/mL. Use FTIR spectrometry to confirm if levamisole is present if you suspect a false positive. For more information on possible interfering substances, please see the BTNX MTS Product Insert.

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Test Strip	Sample Type/Form	Starting Dilution	Result	Next Step	Final Interpretation
FTS Cut off: 200 ng/mL ≥ 5 mg	Suspected Opioid Powder, Pill	5 mg / 5 mL	**+**	No Further Dilution Needed	Positive
			–	No Further Dilution Needed	Negative
	Suspected Non-Opioid Powder, Pill	5 mg / 5mL	**+**	**Possible False +, Dilute to 30 mL (5 mg / 30 mL), Retest***	Positive at 5 and 30mL = **+** Positive at 5 and Negative at 30mL = **–**
			–	No Further Dilution Needed	True Negative
FTS Cut off: 200 ng/mL ≤ 1 mg	Suspected Opioid Residue, Cooker/Cotton	1 mg / 1mL	**+**	No Further Dilution Needed	Positive
			–	No Further Dilution Needed	Negative
	Suspected Non Opioid Residue, Cooker, Pipe	1 mg / 1mL	**+**	**Possible False +, Dilute to 5 mL (1 mg / 5 mL), Retest***	Positive at 1 and 5mL = **+** Positive at 1mL and Negative at 5mL = **–**
			–	No Further Dilution Needed	Negative
BTS Cut off: 300 ng/mL	Suspected Benzo Part / whole pill	5 mg / 2 mL	**+**	No Further Dilution Needed	Positive
		5 mg / 2 mL	**–**	No Further Dilution Needed	Negative
XTS* NTS MTS*** *Cut off: 1,000 ng/mL **Cut off: 2,000 ng/mL	Suspected Opioid Powder, Pill	10mg / 1mL	**+**	**Dilute to 5 mL (10 mg / 5 mL), Retest***	Positive at 1 and 5 mL = **+** Positive at 1 mL and Negative at 5 mL = Inconclusive
			–	**No Further Dilution Needed if - Indicator Red line is strong** **If the second line indicating - is faint, add more sample (5mg or 1 microscoop), Retest***	Two strong red lines = **-** Faint lines or different results at various concentrations = Inconclusive.

***Retest* - False positives are likely due to cross-reacting substances. For example, if testing a suspected meth sample that is positive at 5mL on an FTS, dilute further to decrease the concentration. FTS are highly sensitive and will detect fentanyl at this dilution. If there is suspicion of a known cross-reactive substance (e.g. Lidocaine on XTS) then consider running the test strip again with more water. If you had run a sample at 10 mg / 1 mL, add another 4 mL water and retest at 10 mg / 5 mL.**
Questions? Please contact: maddsbrandeis@gmail.com

Sensitivities, Concentrations, False Positives, and Cross-Reacting Substances Overview

Sensitivities of Test Strips:
FTS - Cut off: 200 ng/mL
BTS - Cut off: 300 ng/mL
XTS - Cut off: 1,000 ng/mL
NTS - Cut off: 2,000 ng.mL
MTS - Cut off: 1,000 ng/mL

Concentration is being used to refer to the amount of sample in a given amount of solvent (in this case water).

- **More concentrated solution is needed for less sensitive strips (Xylazine, Medetomidine, Nitazene)**
  - 10 mg / 1 mL
- **Less concentrated solution is needed for more sensitive strips (Fentanyl, Benzo)**
  - 5 mg / 5 mL or 5 mg / 2 mL

FTS are very sensitive and don't require a large amount of sample for an accurate result while XTS are less sensitive and require a more concentrated solution.

False Positives caused by cross-reacting substances:

Running test strips at a very concentrated solution is intended to pick up very low levels of the intended substance but poses the risk of a cross reacting substance (e.g. Methamphetamine on FTS or Lidocaine on XTS) tripping a false positive result).

Diluting the solution further will reduce the likelihood that the positive result is due to the cross-reactive substance. A solution of 2 mg / 1 mL is extremely unlikely to produce a false positive. However, diluting the solution will increase the possibility of a false negative result if the target substance is at a very low concentration for the less sensitive strips.

FTS False Positives:

- Methamphetamine, MDMA, and diphenhydramine are known to cause false positives with FTS at the 5 mL concentration. Diluting to 30 mL will most likely remove the false positive. If testing a suspected meth sample that is positive at 5mL, or if you receive an unexpected positive result on a sample suspected to be a non-opioid, dilute to 30 mL and retest.

XTS False Positives:

- Lidocaine is known to cause false positives with XTS at the 1 mL concentration, diluting to 5 mL will most likely remove the possibility of a false positive. The most recent version of xylazine test strips (2.0) released by BTNX report the cross-reactivity risk with lidocaine has been removed.

What to do if test strip results at different concentrations contradict one another:

- If a test strip is positive at the original concentrated solution but negative at the more dilute concentration, the result should be reported as either negative or inconclusive, depending on additional/accompanying information.
  - If the FTIR results indicate the presence of a known cross-reactive substance (e.g. Methamphetamine or Lidocaine) and the more concentrated solution is positive and then the less concentrated solution is negative, then the test strip result should be reported as negative.

Inconclusive Test Strip Results:

- If test strip results come back inconclusive, the technician should use their best judgement to determine how to enter the result into StreetCheck. Then, the technician should add their thoughts and details on the inconclusive result in the notes section with the test strip entry.