

# Benzodiazepine Test Strips

## Purpose

BTNX benzodiazepine test strips (BTS) are immunoassay strips that detect the presence of benzodiazepines in drug samples. BTS are a useful tool in drug checking services because they can detect benzodiazepines in concentrations below the limit of detection of FTIR spectroscopy.<sup>1</sup> [Results from a BTS study](#) conducted by the BCCSU, in collaboration with Health Canada (DAS), on samples collected in B.C. demonstrated higher sensitivity to trace amounts of benzodiazepines than FTIR spectroscopy.<sup>1,2</sup>

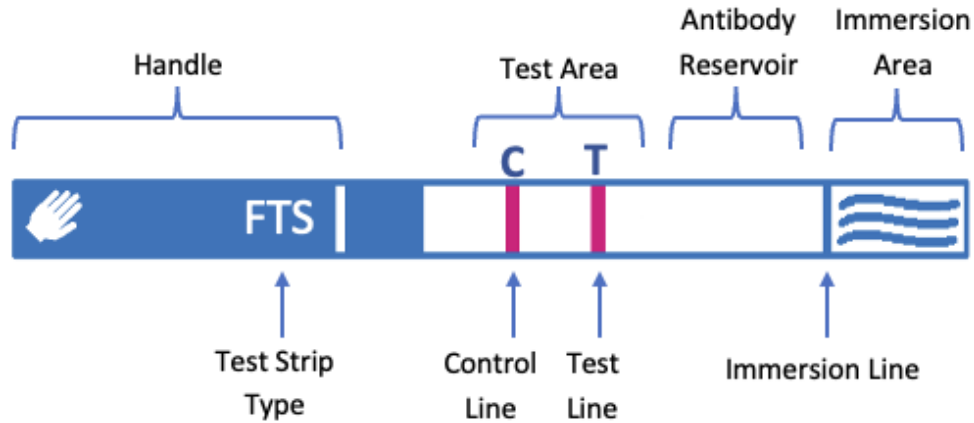
## Scope

Below is the step-by-step procedure for using BTS as part of FTIR drug checking services. A combined test strip procedure is recommended for an efficient workflow that minimizes the amount of drug required for testing. Additional test strip procedures, including fentanyl test strips ([FTS](#)), medetomidine test strips ([MTS](#)), and xylazine test strips ([XTS](#)) are available on our [website](#).

This procedure is intended for drug checking staff using test strips, who are expected to be familiar with [safe handling](#), [drug crushing](#), and [disposal](#) procedures prior to use.

## Understanding Test Strips

When the test strip is dipped into a solution, the liquid moves up the strip and passes through a band of antibodies, which are proteins that bind to target substances. The antibodies are dyed, and as they move up the strip, they bind to the control and test lines, as shown below. The control line must appear for the test to be valid. However, if the target substance (i.e., benzodiazepines) is present, the antibodies will attach to it instead, and the test line will not appear.<sup>3</sup>

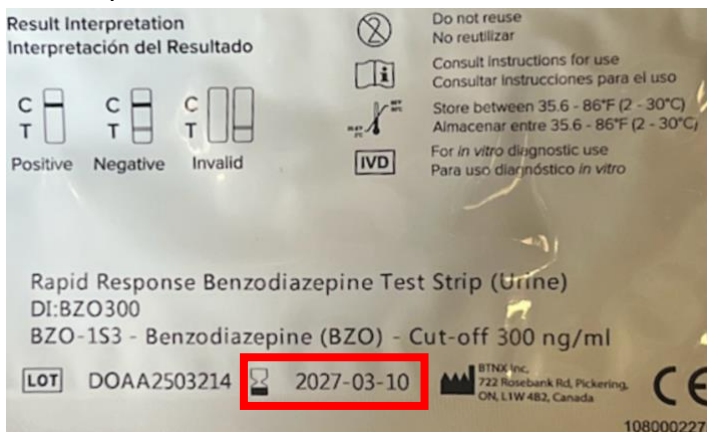


## Setup

Before starting, ensure a clear work area on an impermeable surface where the technician will not be bumped by passersby. Gather the following supplies:

PPE	Cleaning Supplies	Testing Equipment
<ul style="list-style-type: none"> <li>Nitrile gloves</li> <li>Face mask</li> </ul>	<ul style="list-style-type: none"> <li>Alcohol swabs</li> <li>Paper towels</li> </ul>	<ul style="list-style-type: none"> <li>1.5 - 2 ml microcentrifuge tube</li> <li>Microscope (in testing package)</li> <li>Drug crushing device</li> <li>Tap water</li> <li>Benzodiazepine test strip</li> </ul>

Check that the test strip has been stored correctly, the package is not punctured, and the expiry date has not passed (example below). Do not use damaged, opened, or expired test strips, as their results may be invalid.



Expiry date on the test strip package.



Microcentrifuge tubes.

## Procedure

### Preparation

1. Put on disposable gloves and (optional) face mask.
2. Ensure the sample is thoroughly mixed. If not, [crush sample according to procedure](#).

### Prepare Solution

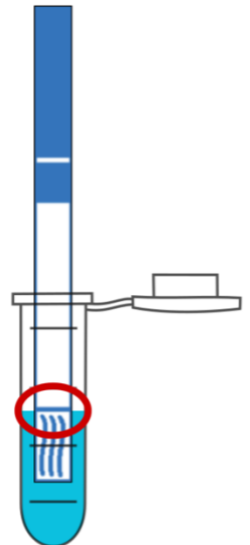
#### Microcentrifuge Tube

3. Add **1-2 mg** of sample to the tube
4. Add **1 ml** of water to the same tube
5. Close and shake for **30 seconds** to dissolve

*Note: Benzodiazepines are difficult to dissolve. Ensure samples are shaken well.<sup>4</sup>  
Microcentrifuge tubes are recommended for co-testing strips.*

### Test Sample

6. Dip the BTS to the immersion line (right-diagram) and absorb for **15 seconds**.
7. Set the BTS down on a non-absorbent surface (tip: use the package). Be mindful of cross-contamination. Wait **2 minutes** before reading the results.
8. Assess test strip under a direct, bright light. If the result appears positive at first, wait a full 5 minutes in case a faint test line shows. Do not interpret after 10 minutes (invalid).



### Clean Workspace

9. [Dispose of solution](#) in clumping cat litter or an activated charcoal disposal pouch.
10. Clean work area and tools using isopropyl alcohol wipes, or if available, follow your worksite's cleaning protocols.
11. Dispose of all testing materials, including the used test strip and gloves.

## Interpreting Test Results

### 1. Positive Result

A positive test strip result (control-line only) indicates that a benzodiazepine is **detected**.<sup>5</sup>



*Considerations:*

- If result is unexpected, consider repeating the test to confirm (e.g., test-positive stimulant; re-test).

### 2. Negative Result

A negative test strip result (both lines) indicates that a benzodiazepine is **not detected**.<sup>5</sup>



*Considerations:*

- A faint test line still means a negative result, and does not indicate a low concentration of a benzodiazepine.
- Test lines are often faint and are best interpreted under a direct, bright light. If the result is unclear at first, wait a full 5 minutes in case a faint test line shows. Do not interpret after 10 minutes (invalid).
- False-negative results are common with certain benzodiazepines (e.g., ethylflualprazolam, etizolam).
- The portion of the sample tested with BTS may not contain benzodiazepines, but the larger sample may (aka. "hot-spots"). See the [chocolate chip cookie effect](#).
- The sample tested may not have been crushed and mixed thoroughly enough to fully dissolve in the solution. See the [drug crushing procedure](#).
- Benzodiazepines may still be present below the [BTS detection threshold](#).
- If result is unexpected, consider repeating the test to confirm (e.g., expected a benzodiazepine but test-negative; increase concentration and re-test).

### 3. Invalid Result

If the control line does not appear, the test is **invalid**.<sup>5</sup>



*Invalid test results can occur from:*

- Testing substances that are not compatible with test strips (e.g., alcohol).
- Saturating the result area (above the immersion line).
- Damaged test strip packaging, introducing humidity.
- Damaged test strip due to bending.
- Improper handling or storage.
- Expired test strips.

*Re-testing:*

1. Repeat the test using an unexpired test strip from an intact package.
2. Carefully conduct the test to rule out user error.
3. Ensure the test strip is being interpreted according to package instructions

## Unexpected Performance

Test strips can sometimes perform unexpectedly, providing results that are either very unlikely or known to be false. This can be due to unknown substance cross-reactivity or manufacturing errors that may affect a number of test strips in a batch (“bad-batch”). Re-test at least once with a new test strip if the result is unexpected. If the unexpected result is consistent, then document as follows:

1. Take a photograph of the used test strips with unexpected results.
2. Record the 10-character lot code(s) (e.g., DOA2204278).
3. Write a brief description of how the test strip performance was unexpected.
4. Send the above items in an email to notify BTNX ([support@btnx.com](mailto:support@btnx.com)) and BCCSU Drug Checking Program ([drugchecking@bccsu.ubc.ca](mailto:drugchecking@bccsu.ubc.ca)).

## Additional Resources

- [BTNX Rapid Response Benzodiazepine Test Strips \(Liquid/Powder\) – 100 Tests](#)
- [Pursell et al. \(2021\). \*Potent sedatives in opioids in BC: Implications for resuscitation, and benzodiazepine and etizolam withdrawal.\*](#)
- [Toward the Heart: Etizolam in BC's Illicit Drug Market](#)

## References

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