



Clinical Update:

MAKING SENSE OF BENZODIAZEPINE RESULTS: CLINICAL AND PRACTICAL IMPLICATIONS OF POCT VS. DEFINITIVE TESTING

After reviewing this Clinical Update, participants will be able to:

1. Describe key analytical limitations of point-of-care testing (POCT) benzodiazepine immunoassays, particularly for lorazepam, clonazepam metabolites, alprazolam metabolites, and non-traditional benzodiazepines. Highlight key safety considerations, including new or emerging substances and interpretation of recent lab results.
2. Compare POCT results with definitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) findings and explain how discordant results may influence adherence assessment, risk evaluation, and clinical decision making.
3. Identify clinical scenarios in which definitive LC-MS/MS benzodiazepine testing provides added value, such as suspected non prescribed use, polysubstance risk, or patient presentations inconsistent with POCT findings.
4. Apply practical strategies for incorporating definitive LC-MS/MS testing into patient care, including when to consider additional testing based on clinical judgment or limitations of immunoassay performance.

Overview

Point-of-care (POC) immunoassays for benzodiazepines are widely used in outpatient, inpatient, and behavioral health settings because they provide rapid, qualitative results at the bedside or in the clinic. However, these assays were developed around older benzodiazepine chemistries and are not optimized for many commonly prescribed agents or newer designer compounds.¹⁻⁴

Recent surveillance and toxicology studies have documented an evolving landscape of designer benzodiazepines, including bromazolam and related analogues, which are increasingly present in the illicit supply and often co-detected with other high-risk substances such as fentanyl.^{1-3,5-9} At the same time, benzodiazepine prescribing and overdose mortality in the United States have increased over the past several decades, underscoring the need for accurate monitoring strategies.⁵

Recent internal data comparing POC benzodiazepine immunoassay results with paired definitive liquid chromatography–tandem mass spectrometry (LC-MS/MS) testing highlight a substantial rate of false-negative screens, particularly for lorazepam, clonazepam metabolites, alprazolam metabolites, and several non-traditional or designer benzodiazepines.¹⁵⁻¹⁷ These discrepancies can directly influence diagnostic reasoning, adherence assessment, and patient safety.

This Clinical Update reviews key findings from that comparison, discusses the clinical implications of discordant results, and offers practical suggestions for when and how to incorporate definitive testing into routine care. A brief section also addresses how these same data can help inform conversations about appropriate test selection and utilization with health plans.

Background

Benzodiazepines remain commonly prescribed for anxiety disorders, insomnia, seizure disorders, and as adjunctive therapy in various psychiatric and neurologic conditions. Non-prescribed benzodiazepine use, including high-potency designer analogues, has meanwhile increased in certain populations and is frequently implicated in polysubstance overdose.^{1-5,8,9}



Routine monitoring for benzodiazepine use is important for several reasons:

- Verification of adherence to a treatment plan
- Detection of undisclosed use that may increase risk (e.g., in combination with opioids, alcohol, or other sedatives)
- Support for treatment decisions in behavioral health and addiction medicine

POC immunoassays play an important role by offering rapid turnaround. However, they have well-recognized limitations:

- Antibodies are typically targeted toward oxazepam-like structures and a limited subset of benzodiazepines.^{1,3,4}
- Cross-reactivity is poor or highly variable for lorazepam, clonazepam metabolites, alprazolam metabolites, and many designer benzodiazepines.^{1-4,8,9}
- Results are qualitative and can be affected by cutoff concentrations, timing of last dose, urine dilution, and metabolite patterns.¹⁰

Definitive LC-MS/MS testing, by contrast, identifies specific parent drugs and metabolites at much lower concentrations and can include broader analyte coverage, including novel or emerging agents that are not reliably detected by immunoassay.^{3,4}

Study Overview: POCT vs. Definitive Benzodiazepine Results

The internal analysis conducted by Aegis evaluated samples that had both:¹⁵⁻¹⁷

- A POC benzodiazepine immunoassay result, and
- A paired definitive LC-MS/MS benzodiazepine panel result from the same patient encounter.

Samples were selected based on prior definitive testing that identified one or more designer benzodiazepines in healthcare-submitted urine specimens, reflecting real-world use in populations at risk for non-prescribed substance exposure.^{15,17}

The primary objectives of the comparison were to:

1. Characterize how often the POC benzodiazepine screen was negative when the definitive test identified one or more benzodiazepines (i.e., false-negative immunoassay results).¹⁵⁻¹⁷
2. Identify which specific benzodiazepines were most frequently missed by immunoassay.¹⁵
3. Consider how these discordances might influence clinical decision-making in real-world settings.^{10,15}

Key Findings

1. Negative POCT Does Not Reliably Exclude Benzodiazepine Exposure

Across the dataset, there was a clinically meaningful subset of encounters in which the POC benzodiazepine screen was negative despite definitive LC-MS/MS identifying one or more benzodiazepine analytes. Compounds frequently involved in these discordant pairs included lorazepam, clonazepam (via 7-aminoclonazepam), alprazolam metabolites, and multiple designer benzodiazepines. These findings highlight a longstanding and well-documented limitation of immunoassay cross-reactivity for several modern benzodiazepines.

2. Performance of POCT in the Designer Benzodiazepine (DBZD) Cohort

In a cohort of 48 urine samples known to contain designer benzodiazepines on definitive LC-MS/MS, only 13 samples (27%) were positive for benzodiazepines on POCT, while 32 (66%) were negative and 3 samples produced discrepant results between POCT devices. Among samples with negative or discrepant POCT findings (n=35), 30 samples (63%) had *only* designer benzodiazepines present on definitive testing, without any detectable



prescription benzodiazepine signal. Importantly, if only unexpected POCT benzodiazepine results had been sent for definitive testing that included designer benzodiazepines, non-prescribed/illicit benzodiazepine use would have been identified in only 15 patients (31%), despite all 48 individuals in this cohort having DBZD exposure on definitive testing. This demonstrates that relying solely on POCT-driven reflex testing would miss approximately two-thirds of DBZD-positive cases.

3. Clinical Scenarios Most Impacted by Discordant Results

The discordance between POCT and definitive benzodiazepine testing is especially consequential in real-world scenarios such as:

- Medication adherence monitoring, where false-negative POCT results can incorrectly imply non-adherence or diversion.
- Risk assessment in patients receiving opioids, where unrecognized benzodiazepine exposure may compound sedation and respiratory-depression risk.
- Evaluation of unexplained sedation or intoxication, where designer benzodiazepines are increasingly implicated but not detected by immunoassay.
- Behavioral health and addiction treatment, where the presence or absence of benzodiazepines materially influences treatment planning, safety assessment, and level-of-care decisions.

Discussion

The observed pattern of discordance between POCT and definitive benzodiazepine testing is consistent with both the pharmacology of modern benzodiazepines and the design of commonly used immunoassays. In this DBZD-positive cohort of 48 urine samples (each known to contain at least one designer benzodiazepine on definitive testing), only 13 samples (27%) were positive for benzodiazepines on POCT, while 32 (66%) were negative and 3 had discrepant results between the two POCT cup brands. This means that roughly three-quarters of DBZD-positive samples did not yield a straightforward positive result on commonly used benzodiazepine POCT devices.

From a clinical standpoint, the most striking observation is not just that POCT can miss designer benzodiazepines, but that it does so frequently even when benzodiazepine exposure is definitively known to be present. In this study, all 48 samples had at least one DBZD identified by LC-MS/MS; however, a clinician relying on POCT alone would have seen a “reassuring” negative benzodiazepine screen in the majority of cases. For the 35 samples with negative or discrepant POCT results (32 negative, 3 discrepant), 30 (63%) had only designer benzodiazepines present on definitive testing.

This has several practical implications:

- Under-recognition of non-prescribed/illicit benzodiazepine use.
 - If a typical “test only when POCT is unexpected” strategy were applied, and only unexpected POCT benzodiazepine results were sent for definitive testing that included DBZDs, non-prescribed/illicit benzodiazepine use would have been recognized in only 15 patients (31%) in this cohort, even though all 48 actually had DBZD exposure. In other words, approximately two-thirds of DBZD-positive patients would have remained unidentified.
- False reassurance from negative POCT results.
 - Many of the negative POCT results were clinically “expected” based on prescription records (no Rx benzodiazepine) or patient-reported histories. Yet definitive testing revealed DBZD exposure in every case. For high-risk individuals, including those with substance use disorders or polysubstance exposure, a negative benzodiazepine POCT should not be interpreted as absence of benzodiazepine-related risk when clinical suspicion is present.
- Complex exposure patterns that POCT cannot characterize.



- Several samples contained multiple DBZDs, and some had both an Rx benzodiazepine and one or more DBZDs. These mixed exposure patterns are particularly relevant in patients on opioids or other CNS depressants, where cumulative sedative burden and overdose risk are high. Immunoassay screens cannot distinguish which specific benzodiazepines are present, nor quantify the number of distinct agents contributing to the clinical picture.

Collectively, the numerical findings from this study reinforce a key principle: POCT immunoassays are fundamentally limited in their ability to detect designer benzodiazepines and many non-traditional benzodiazepine exposures, and a negative POCT result should not be used to rule out benzodiazepine involvement in patients at elevated risk. In clinical scenarios where benzodiazepine identification will meaningfully influence management, such as evaluating unexplained sedation, reassessing risk in patients receiving opioids, or monitoring complex behavioral-health populations, definitive LC-MS/MS testing provides a more reliable assessment of exposure.

These data also illustrate why a purely reactive testing strategy, like only sending “unexpected” POCT results for definitive confirmation, may be insufficient in populations where DBZDs are circulating. In such settings, a more proactive approach to definitive testing, guided by clinical risk and presentation rather than POCT result alone, is likely to better align with patient safety and the realities of the contemporary drug supply.

Practical Considerations for Clinicians

Based on the comparison of POCT and definitive benzodiazepine testing, the following strategies may help integrate these modalities more effectively:

1. Clarify the clinical question up front
 - If the key question is whether the patient is taking a specific prescribed benzodiazepine, or whether benzodiazepines could be contributing to the current presentation, immunoassay alone may not be sufficient, particularly in higher-risk or diagnostically complex situations.^{10,11}
2. Recognize which benzodiazepines are poorly detected by POCT
 - Maintain a high index of suspicion for false-negative POCT results in patients prescribed lorazepam, clonazepam, or alprazolam, and in populations where designer benzodiazepine exposure is possible.^{1-4,8,9,15}
3. Use definitive testing strategically
 - Consider ordering definitive LC-MS/MS benzodiazepine testing when:
 1. POCT results are inconsistent with the clinical picture.
 2. Confirming adherence or non-use will meaningfully change treatment decisions.
 3. Patients are at elevated risk due to polypharmacy (e.g., concurrent opioids, gabapentinoids, or other CNS depressants).^{5,10-14}
 4. There is concern for designer or non-prescribed agents that are not reliably detected by immunoassay.^{1-4,8,9,15}
4. Interpret negative POCT results cautiously
 - Document when clinical suspicion remains high despite a negative POCT and when definitive testing is pursued to resolve that discrepancy.¹⁰
5. Communicate findings to patients
 - Explaining that different tests have different capabilities can support shared decision-making and build trust when additional testing is recommended, consistent with principles from addiction-medicine and chronic pain guidelines.¹⁰⁻¹⁴



Conclusion

The comparison of POC benzodiazepine immunoassays with definitive LC-MS/MS testing underscores a key message: rapid immunoassay screens are useful tools, but they cannot stand alone in many modern benzodiazepine monitoring scenarios. False-negative POCT results are common for several widely used benzodiazepines and for emerging designer compounds, with real implications for adherence assessment, risk evaluation, and patient safety.^{1-5,8,9,15-17}

Clinicians should:

- Understand which benzodiazepines are poorly detected by immunoassay,^{1-4,8,9}
- Use definitive testing thoughtfully when results will influence care,¹⁰⁻¹⁴ and
- Interpret negative POC screens with caution when the clinical picture suggests benzodiazepine exposure.^{10,11,15}

By integrating both testing modalities in a targeted, clinically driven way, and by clearly communicating the rationale for test selection, providers can better support safe prescribing, effective treatment planning, and informed discussions with patients and payers alike.¹⁰⁻¹⁴

Please call our clinical scientists at 1-877-552-3232 if you require additional information.

NOTICE: The information above is intended as a resource for health care providers. Providers should use their independent medical judgment based on the clinical needs of the patient when making determinations of who to test, what medications to test, testing frequency, and the type of testing to conduct.

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Highlights & Clinical Takeaways

- Negative benzodiazepine POCT results do *not* reliably exclude exposure. Immunoassay screens frequently miss commonly prescribed benzodiazepines (e.g., lorazepam, clonazepam, alprazolam metabolites) and designer benzodiazepines.
- Designer benzodiazepines are often invisible to POCT. In a DBZD-positive cohort, approximately two-thirds of samples with confirmed exposure were negative on point-of-care immunoassay testing, creating false reassurance.
- Reliance on POCT-driven reflex testing can miss most nonprescribed benzodiazepine use. If definitive testing is ordered only when POCT results are “unexpected,” the majority of DBZD exposures may go undetected.
- Discordant results have real clinical consequences. False-negative POCT findings can affect adherence assessments, underestimate polysubstance risk (especially with opioids), and delay recognition of benzodiazepine-related sedation or overdose risk.
- Definitive LC-MS/MS testing provides a more complete risk assessment. Mass-spectrometry–based testing identifies specific benzodiazepines and analogues at lower concentrations and better reflects the modern drug supply.

Recommendations for Clinicians

- Interpret negative benzodiazepine POCT results cautiously, particularly in patients prescribed lorazepam, clonazepam, or alprazolam, and in populations at risk for designer benzodiazepine exposure.
- Use definitive LC-MS/MS testing when results will influence care, including situations involving unexplained sedation, opioid co-prescribing, adherence assessment, or suspected nonprescribed substance use.
- Do not rely on immunoassay results alone to assess safety risk in patients with polysubstance exposure or behavioral health complexity.
- Align testing strategy with clinical risk, not convenience. In high-risk scenarios, definitive testing is not a secondary step, but rather it is often the appropriate first-line method.