New High-Resolution Targeted Benzodiazepine Screen

Superior Sensitivity and Specificity to Evaluate Adherence to Prescribed Benzodiazepine Therapy

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Disclosures

• None

Objectives

• Describe the clinical utility of benzodiazepines and understand the limitations of the following urine drug tests used to support adherence monitoring of these medications:
  • Laboratory or point of care-based benzodiazepine immunoassays
  • Mass spectrometry-based targeted screening assays
• Define the metabolic profiles of benzodiazepines commonly prescribed and discuss how to interpret screening/definitive test results
**Benzodiazepines**

- A large class of central nervous system (CNS) depressant medications with useful sedative, hypnotic, anxiolytic, and anticonvulsant properties
- **Types:**
  - Short, Intermediate, and Long-acting benzodiazepines
- **FDA-approved uses:**
  - Generalized anxiety disorders
  - Panic disorders
  - Social phobia
  - Insomnia
  - Status epilepticus/seizures
  - Premedication for anesthetic procedures

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**Benzodiazepines**

- **Mechanism of Action:**
  - Raise levels of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) in the brain
- **Common examples:**
  - Diazepam (Valium)
  - Alprazolam (Xanax)
  - Clonazepam (Klonopin)
  - Lorazepam (Ativan)
- **Class IV Controlled Substance**
- >30% Opioid Overdoses also involved a Benzodiazepine
Screening Assays

Types:
- Traditional screening assays
- Point-Of-Collection Tests (POCT)
- Laboratory-based (commercial immunoassays)
- Targeted screening assays
  - Laboratory-Developed-Tests (LDT) using TOF-MS or other MS/MS analyzers

<table>
<thead>
<tr>
<th>Test</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>POCT</td>
<td>Fastest TAT</td>
<td>Limited sensitivity</td>
</tr>
<tr>
<td></td>
<td>CLIA waived versions available</td>
<td>Higher cutoffs</td>
</tr>
<tr>
<td></td>
<td>Instant result to review/discuss with patient</td>
<td>Limited specificity</td>
</tr>
<tr>
<td></td>
<td>Great if patient resides far from care</td>
<td>Maintain inventory/regulatory compliance</td>
</tr>
<tr>
<td></td>
<td>Good for high-risk patient</td>
<td>Higher cost</td>
</tr>
<tr>
<td>Immunoassay-Lab based</td>
<td>Automated</td>
<td>Limited sensitivity</td>
</tr>
<tr>
<td></td>
<td>CLIA environment</td>
<td>Limited specificity</td>
</tr>
<tr>
<td></td>
<td>Most economic</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Larger test menu</td>
<td></td>
</tr>
<tr>
<td>Targeted screen</td>
<td>Better sensitivity</td>
<td>Limited availability</td>
</tr>
<tr>
<td></td>
<td>Better specificity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Broader test menu</td>
<td></td>
</tr>
</tbody>
</table>

Imunoassays & Cross-Reactivity Issues

- Common urine Benzodiazepine immunoassay targets:
  - Oxazepam or Nordiazepam
- Concentrations required to trigger a “positive” Benzodiazepine result:

<table>
<thead>
<tr>
<th>Drug</th>
<th>100 ng/mL cutoff</th>
<th>% Cross reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-aminoclonazepam</td>
<td>144 ng/mL</td>
<td>69%</td>
</tr>
<tr>
<td>α-hydroxyalprazolam</td>
<td>118 ng/mL</td>
<td>84%</td>
</tr>
<tr>
<td>Lorazepam</td>
<td>103 ng/mL</td>
<td>62%</td>
</tr>
<tr>
<td>Lorazepam glucuronide</td>
<td>19,615 ng/mL</td>
<td>0.5%</td>
</tr>
<tr>
<td>Midazolam</td>
<td>168 ng/mL</td>
<td>60%</td>
</tr>
<tr>
<td>α-hydroxymidazolam</td>
<td>140 ng/mL</td>
<td>71%</td>
</tr>
<tr>
<td>Temazepam</td>
<td>145 ng/mL</td>
<td>69%</td>
</tr>
<tr>
<td>Temazepam glucuronide</td>
<td>&gt;20,000 ng/mL</td>
<td>0.8%</td>
</tr>
</tbody>
</table>
Clinical Case Study #1

- Case History:
  - 55 year old female
- Medical History:
  - Anxiety disorder
- Medications:
  - Lorazepam (Ativan)
- Clinical Evaluation:
  - Anxiety well controlled
  - Orders urine drug testing (UDT) to monitor compliance

Case #1 Continued

- Routine Lab or POCT-based Urine Immunoassay results:
  - January 2019

<table>
<thead>
<tr>
<th>Immunoassay Screening Test</th>
<th>Urine Cutoff</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamine/Methamphetamine</td>
<td>500 ng/mL</td>
<td>Negative</td>
</tr>
<tr>
<td>Barbiturates</td>
<td>200 ng/mL</td>
<td>Negative</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>100 ng/mL</td>
<td>Negative</td>
</tr>
<tr>
<td>Cocaine Metabolite</td>
<td>150 ng/mL</td>
<td>Negative</td>
</tr>
<tr>
<td>Phencyclidine (PCP)</td>
<td>25 ng/mL</td>
<td>Negative</td>
</tr>
<tr>
<td>THC (Marijuana)</td>
<td>50 ng/mL</td>
<td>Negative</td>
</tr>
<tr>
<td>Opiates</td>
<td>300 ng/mL</td>
<td>Negative</td>
</tr>
</tbody>
</table>
Is the Patient Compliant Based on the Urine Test Results?

- Patient prescribed Lorazepam
- Patient Urine Benzodiazepine Immunoassay Result:

<table>
<thead>
<tr>
<th>Screening Test</th>
<th>Urine Cutoff</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzodiazepine</td>
<td>100 ng/mL</td>
<td>Negative</td>
</tr>
</tbody>
</table>

- Remember cross-reactivity w/ Immunoassay:

<table>
<thead>
<tr>
<th>Drug</th>
<th>100 ng/mL cutoff</th>
<th>% Cross reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lorazepam</td>
<td>163 ng/mL</td>
<td>62%</td>
</tr>
<tr>
<td>Lorazepam glucuronide</td>
<td>19,615 ng/mL</td>
<td>0.5%</td>
</tr>
</tbody>
</table>

- Lorazepam appears in the urine largely as the glucuronidated conjugate

Simplified Benzodiazepine Metabolism

- Alprazolam (Xanax)
- Chlordiazepoxide (Librium)
- Clorazepate (Tranxene)
- Halazepam (Paxipam)
- Prazepam (Centrax)
- Medazepam (Nortriptyline)
- Clonazepam (Klonopin)
- Estazolam (Procon)
- Flurazepam (Dalmane)
- Lorazepam (Ativan)
- Midazolam (Versed)
- Triazolam (Halcion)
- Diazepam (Valium)
- Temazepam (Restoril)
- 7-aminolozepam
- 4-hydroxyflurazepam
- N-hydroxyethylflurazepam
- Lorazepam glucuronide
- 7-amino lorazepam
- 4-hydroxy estazolam
- N-hydroxy ethyl flurazepam
- Lorazepam glucuronide
- 7-aminolozepam
- 4-hydroxyflurazepam
- N-hydroxyethylflurazepam
- Lorazepam glucuronide
- 7-aminolozepam
- 4-hydroxyflurazepam
- N-hydroxyethylflurazepam
- Lorazepam glucuronide
Case #1 Continued; Physician Adds On Order for Benzodiazepine Confirmation Test

• Benzodiazepine LC-MS/MS Confirmation, Urine (BENZU)

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Urine Detection Limit</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-NH-Clonazepam</td>
<td>100 ng/mL</td>
<td>&lt;100</td>
</tr>
<tr>
<td>7-NH-Flunitrazepam</td>
<td>100 ng/mL</td>
<td>&lt;100</td>
</tr>
<tr>
<td>Alpha-OH-Alprazolam</td>
<td>100 ng/mL</td>
<td>&lt;100</td>
</tr>
<tr>
<td>Alpha-OH-Triazolam</td>
<td>100 ng/mL</td>
<td>&lt;100</td>
</tr>
<tr>
<td>OH-ethyl-flurazepam</td>
<td>100 ng/mL</td>
<td>&lt;100</td>
</tr>
<tr>
<td>Lorazepam</td>
<td>100 ng/mL</td>
<td>8,250 ng/mL</td>
</tr>
<tr>
<td>Nordiazepam</td>
<td>100 ng/mL</td>
<td>&lt;100</td>
</tr>
<tr>
<td>Oxazepam</td>
<td>100 ng/mL</td>
<td>&lt;100</td>
</tr>
<tr>
<td>Temazepam</td>
<td>100 ng/mL</td>
<td>&lt;100</td>
</tr>
</tbody>
</table>

• Conclusion:
  - Patient is taking Lorazepam (Ativan)

Mayo Clinic’s High Resolution Targeted Benzodiazepine Screen (TABSU)
### High-Resolution Targeted Benzodiazepine Screen

**TABSU / Targeted Benzodiazepine Screen, Urine**

- **Features and Benefits:**
  - Uses high-resolution accurate mass spectrometry to identify 27 different benzodiazepines and/or metabolites where immunoassays are not adequate
  - Lower detection limits (improved sensitivity)

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### Table 4: Summary of Evidence-Based LMPG Recommendations

<table>
<thead>
<tr>
<th>#</th>
<th>Recommendation</th>
<th>Grading</th>
<th>Target Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Qualitative definitive tests should be used over immunoassays since they are more effective at identifying relevant over-the-counter medications, prescribed and non-prescribed drugs, and illicit substances in pain management patients.</td>
<td>A, II</td>
<td>X</td>
</tr>
</tbody>
</table>

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2017 AACC Academy LMPG
Evidence-based Recommendation: Definitive Testing

Table 4: Summary of Evidence-Based LMPG Recommendations
## Drugs Detected in the High-Resolution Targeted Benzodiazepine Screen

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Cutoff</th>
<th>Analyte</th>
<th>Cutoff</th>
<th>Analyte</th>
<th>Cutoff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alprazolam</td>
<td>10 ng/mL</td>
<td>Nordiazepam</td>
<td>10 ng/mL</td>
<td>Oxazepam</td>
<td>10 ng/mL</td>
</tr>
<tr>
<td>Alpha-Hydroxyalprazolam</td>
<td>10 ng/mL</td>
<td>Flunitrazepam</td>
<td>10 ng/mL</td>
<td>Oxazepam Glucuronide</td>
<td>50 ng/mL</td>
</tr>
<tr>
<td>Alpha-Hydroxyalprazolam Glucuronide</td>
<td>50 ng/mL</td>
<td>7-aminoflunitrazepam</td>
<td>10 ng/mL</td>
<td>Prazepam</td>
<td>10 ng/mL</td>
</tr>
<tr>
<td>Chlordiazepoxide</td>
<td>10 ng/mL</td>
<td>Flurazepam</td>
<td>10 ng/mL</td>
<td>Temazepam</td>
<td>10 ng/mL</td>
</tr>
<tr>
<td>Clozapam</td>
<td>10 ng/mL</td>
<td>2-Hydroxy Ethyl Flurazepam</td>
<td>10 ng/mL</td>
<td>Temazepam Glucuronide</td>
<td>50 ng/mL</td>
</tr>
<tr>
<td>N-Desmethylclozapam</td>
<td>200 ng/mL</td>
<td>Lorazepam</td>
<td>10 ng/mL</td>
<td>Triazolam</td>
<td>10 ng/mL</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>10 ng/mL</td>
<td>Lorazepam Glucuronide</td>
<td>50 ng/mL</td>
<td>Alpha-Hydroxy Triazolam</td>
<td>10 ng/mL</td>
</tr>
<tr>
<td>7-aminoclonazepam</td>
<td>10 ng/mL</td>
<td>Midazolam</td>
<td>10 ng/mL</td>
<td>Zolpidem</td>
<td>10 ng/mL</td>
</tr>
<tr>
<td>Diazepam</td>
<td>10 ng/mL</td>
<td>Alpha-Hydroxy Midazolam</td>
<td>10 ng/mL</td>
<td>Zolpidem Phenyl-4-Carboxylic acid</td>
<td>10 ng/mL</td>
</tr>
</tbody>
</table>

## High-Resolution Targeted Benzodiazepine Screen: TABSU / Targeted Benzodiazepine Screen, Urine

- **Features and Benefits:**
  - Uses high-resolution accurate mass spectrometry to identify 27 different benzodiazepines and/or metabolites where immunoassays are not adequate
  - Lower detection limits (improved sensitivity)
  - Improved test utilization without compromising turn-around-times
  - Significantly reduced need for confirmatory testing required with traditional immunoassay screens
  - Ability to detect “spiked” samples
  - Available with enhanced reports with interpretative comments
Example of Enhanced Report

Targeted Benzodiazepine Screen, U

Controlled Substance Monitoring Panel (CSMP)

- Profile
  - Adulterant Testing:
    - Creatinine, Specific gravity, pH, Oxidant/Nitrite
  - Targeted Opioid & Benzodiazepine Screen:
    - 33 Opioids (parent/metabolites)
    - 27 Benzodiazepines (parent/metabolites)
  - Immunoassay Screens:
    - Amphetamine/Methamphetamine (Cutoff: 500 ng/mL)
    - Barbiturates (Cutoff: 200 ng/mL)
    - Cocaine metabolite (Benzoylcegonine; Cutoff: 150 ng/mL)
    - Phencyclidine (Cutoff: 25 ng/mL)
    - Tetrahydrocannabinol (Cutoff: 50 ng/mL)
Summary

- Objective measures like laboratory tests are needed to:
  - Identify and evaluate recent drug use/abuse
  - Set and monitor clinical goals/expectations
- The new high-resolution targeted benzodiazepine assay:
  - Identifies 27 different benzodiazepines and/or metabolites where immunoassays are not adequate
  - Has lower detection limits (improved sensitivity)
  - Reduces need for confirmatory testing required with traditional immunoassay screens
  - Improves test utilization
  - Available with enhanced reports and interpretative comments
- UDT results need to be interpreted in the context of the test, drug(s) prescribed, specimen type, specimen validity test results, and the patient. Unexpected/unexplained results should be discussed with the patient/laboratory, and additional testing performed if needed
References


