EDUCATIONAL COMMENTARY – TESTING FOR AMPHETAMINES AND RELATED COMPOUNDS

Learning Outcomes

Upon completion of this exercise, participants will be able to:

- list amphetamine-like compounds that may be detected by immunoassay screening tests for amphetamines.
- describe the 3 types of amphetamine immunoassays based on their antibody specificity.
- discuss common causes of false-positive results when testing for amphetamines.

Correct interpretation of testing results for amphetamines and related compounds is dependent on many factors including an understanding of the nomenclature, structure, and metabolism of these compounds. The class of phenethylamine compounds having varying degrees of sympathomimetic activity include amphetamine, methamphetamine, and many other compounds known by several names including amphetamines (as a group), designer drugs often grouped together as ecstasy [3,4-methylenedioxyamphetamine (MDMA); 3,4-methylenedioxyamphetamine (MDA), and 3,4-methylenedioxy-N-ethylamphetamine (MDEA)], and sympathomimetic amines including ephedrine, pseudoephedrine, and phenylpropanolamine found in over-the-counter (OTC) medications.

Amphetamine, a primary amine, and methamphetamine, a secondary amine, have a stereogenic center and have enantiomers or optical isomers designated $d$ (or +) for dextrorotatory and $l$ (or -) for levorotatory. In general, the $d$ isomers are the more physiologically active compounds, but pharmaceutical preparations may consist of either isomer or a mixture of both isomers. When the mixture contains equal concentrations of the 2 enantiomers, it is known as a racemic mixture. The existence of enantiomers for amphetamines creates analytical and interpretative problems.

Immunoassay Screening

The primary screening methods for detecting amphetamines are immunoassays. The structural similarity to amphetamine or methamphetamine of the compounds previously mentioned makes it difficult to produce antibodies specific for amphetamine, methamphetamine, or both. Monoclonal and polyclonal antibodies have been developed and used in amphetamine immunoassays. Monoclonal antibodies result in more defined specificities but not necessarily more selectivity. In general, amphetamines assays can be classified into 3 general types based on antibody specificity:

1. Assays that are highly selective for either amphetamine (and its designer counterpart MDA) or methamphetamine (and its designer drug counterpart MDMA) but not both sets simultaneously.
2. Assays that are able to detect both amphetamine and methamphetamine to varying extent but that also exhibit higher levels of cross-reactivity to the hydroxy amine compounds found in many OTC drugs.

3. Assays that consist of dual assays for amphetamine and methamphetamine with low levels of cross-reactivity to OTC drugs.

Up-to-date cross-reactivity data are typically listed in immunoassay package inserts or may be obtained from each manufacturer. Ideally, laboratories should perform cross-reactivity studies of the most common interfering substances on their reagent/instrument system, but at a minimum they should contact the manufacturer to verify that they have the most recent applicable cross-reactivity information.

Quite often, antibody specificity is influenced by the intended use of the testing. Laboratories performing workplace drug testing desire immunoassays specific for only those compounds (typically amphetamine and methamphetamine) specified in appropriate legislation or contracts. On the other hand, laboratories affiliated with an emergency department (ED) desire immunoassays directed toward the broad spectrum of sympathomimetic amines. This desire is articulated as a recommendation in the National Academy of Clinical Biochemistry’s (NACB) Laboratory Medicine Practice Guidelines (LMPG) that the optimum immunoassays for amphetamines testing in ED patients are those directed toward phenylethyl amines as a class. These guidelines also recommend that the name of the test should be changed from “amphetamines” to “sympathomimetic amines” or “stimulant amines.”

Federally mandated testing currently includes a screening cutoff of 1,000 ng/mL for amphetamines (amphetamine and methamphetamine only) but the Substance Abuse and Mental Health Services Administration (SAMHSA) has proposed to lower these levels to 500 ng/mL and to test also for MDMA (ecstasy).

**False-Positive Immunoassay Results**

Prescription and OTC medications (or metabolites of these medications) reported to interfere with amphetamine immunoassays are listed in the Table. The most common cause of false-positive results with amphetamine immunoassays is cross-reactivity with α-hydroxy amine compounds and other sympathomimetic amines found in many OTC drugs. In order to decrease these false-positive incidents, some laboratories perform a secondary screen utilizing either a different manufacturer’s immunoassay with different cross-reactivity to these compounds or reanalysis using the same immunoassay following chemical reaction to remove interfering compounds such as pretreatment with sodium periodate in a basic solution.
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TABLE. Drugs (and/or Metabolites) Reported To Interfere With Amphetamine Immunoassays.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Drug</th>
<th>Drug</th>
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</thead>
<tbody>
<tr>
<td>Buflomedil</td>
<td>Isoxsuprine</td>
<td>Phenmetrazine</td>
<td>Ranitidine</td>
</tr>
<tr>
<td>Brompheniramine</td>
<td>Labetalol</td>
<td>Phentermine</td>
<td>Ritodrine</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>Mephentermine</td>
<td>Phenypropanolamine</td>
<td>Trimethobenzamide</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>Mexiletine</td>
<td>Promethazine</td>
<td>Tyramine</td>
</tr>
<tr>
<td>Ephedrine</td>
<td>Nylidrin</td>
<td>Propylhexedrine</td>
<td></td>
</tr>
<tr>
<td>Fenfluramine</td>
<td>N-acetylprocainamide</td>
<td>Pseudoephedrine</td>
<td></td>
</tr>
<tr>
<td>Isometheptene</td>
<td>Perazine</td>
<td>Quinicrine</td>
<td></td>
</tr>
</tbody>
</table>

The existence of \(d\) and \(l\) isomers can cause false-positive results when medication containing the \(l\) isomer is ingested. Immunoassays and most gas chromatography–mass spectrometry (GCMS) confirmation procedures do not differentiate these isomers, and it is necessary to perform isomer resolution analysis to determine that a positive result is due to the presence of the \(d\) isomer. This is particularly true for positive methamphetamine results because of the presence of \(l\)-methamphetamine in Vicks inhaler (Procter and Gamble Pharmaceuticals, Cincinnati, OH), which cannot be distinguished from use of illicit methamphetamine \((d\) isomer and racemic mixture depending on method of production) by most GCMS methods. Similarly, patients taking selegiline for Parkinson’s disease will excrete \(l\) methamphetamine and \(l\) amphetamine.

The GCMS isomer resolution procedures using either an optically active chiral column or chiral derivatizing reagents differentiate \(d\) and \(l\) isomers of amphetamine and methamphetamine. The generally accepted interpretation of isomer resolution results is that greater than 80% of the \(l\) isomer is considered consistent with use of legitimate medication, or conversely greater than 20% of the \(d\) isomer (and total concentration above the cutoff) is considered evidence of illicit use.

True-Positive Results

In addition to structurally related and other cross-reacting medications, positive amphetamine results can be obtained when amphetamine, methamphetamine, or medications containing compounds metabolized to amphetamine and/or methamphetamine are ingested. Amphetamine [Dexedrine (GlaxoSmithKline, Research Triangle Park, NC) \((d\)-amphetamine\)], Adderall (Shire US Inc., Wayne, PA ) \((d\) and \(l\)-amphetamine\), etc, and methamphetamine [Desoxyn (Ovation Pharmaceuticals Deerfield, IL) \((d\)-methamphetamine\)] are the active compounds of medications prescribed for narcolepsy, attention deficit disorder, and appetite suppression, and ingestion of these drugs will result in excretion of these compounds in the urine. In addition, compounds known to be metabolized to amphetamine and/or...
methamphetamine include selegiline, amphetamine, benzphetamine, clobenzorex, dimethylamphetamine, ethylamphetamine, famprofazone, fencamine, fenethylline, fenproporex, furfenorex, mefenorex, and prenylamine. Some weight loss or nutritional supplements contain fenproporex and use of these supplements has resulted in detection of d-amphetamine in the urines of users. In January 2006, the Food and Drug Administration (FDA) warned consumers that Brazilian dietary supplements Emagrece Sim and Herbathin (both products produced by Fitoterapicos in Brazil and imported and distributed by Emagrece Sim Laboratories and Herbathin, both of Miami, FL) contain active drug ingredients.

Conclusion
Testing for amphetamine, methamphetamine, and amphetamine-like compounds focuses primarily on detection of amphetamine, methamphetamine, and designer drugs often grouped together as ecstasy (MDMA, MDA, MDEA). The primary concern for immunoassay screening is false-positive results due to cross-reactivity of the reagent antibodies to other sympathomimetic amines, namely the hydroxyl amines ephedrine, pseudoephedrine, and phenylpropanolamine. Periodate treatment of samples prior to immunoassay screening or GCMS confirmation removes the interfering sympathomimetic amines. Another problem with amphetamine testing is the existence of d and l stereoisomers, which are not distinguishable by immunoassay screening and most GCMS confirmation procedures. When reporting and interpreting results of testing for amphetamines in urine, laboratories should be aware of these potential problems, and of the performance characteristics of the assays that they use.

Suggested Reading


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