AACC Online Conference

# The Laboratory's Role in Drug Monitoring for Pain Management

## Analytical Methods II

## Immunoassay

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# Learning Objectives

After this presentation, you should be able to:

1. List the issues affecting immunoassay performance characteristics
2. Define what are immunoassay true positive and false positive results
3. State the reason for performing confirmation testing on immunoassay positive results
4. Define what is an immunoassay false negative result
5. Describe an opiate vs. an opioid

## Screen by immunoassays

- Widely used technology. Can be performed as
  - Point of care testing (POCT)
    - In doctor's office or clinic
    - Rapid turnaround time
    - 'Waived' tests available
  - Laboratory testing; instrument based testing
    - High volume testing possible by automation
    - Longer turnaround time

# What You Need To Know About Immunoassays

1. Immunoassays are qualitative assays
2. Assay immuno-reactivity and accuracy
3. Immunoassays are ‘class’ assays
4. Assay immuno-reactivity for a drug determines drug detection limit
5. Assay immuno-reactivities vary with assay manufacturers
6. Assay Cutoffs

1. Immunoassays are qualitative assays

- Assay 'Cutoff' defines result as positive or negative

  absorbance ≥ calibrator at cutoff = positive

  absorbance < calibrator at cutoff = negative

Opiates assay cutoff = 300 ng/ml of morphine

300 ng/ml = 30,000 ng/ml morphine = positive

299 ng/ml = 0 ng/ml = negative

**Negative does not mean devoid of drug!!**

## 2. Assay Immunoreactivity And Accuracy

- Immunoassay is not 'specific' for one drug
  - Detects all cross-reacting compounds: Result can be true or false positive
    - True Positive: detects member(s) of that class (e.g., methamphetamine by Amphetamines assay)
    - False Positive: Detects drugs not of that 'class' (e.g. buproprion by Amphetamines assay, ofloxacin by Opiates assay)
- Therefore, initial positive result should be confirmed with specific identification

## 3. Immunoassays Are ‘Class’ Assays

- Immunoassay is not ‘specific’ for one drug
  - Detects all cross-reacting members of that ‘class’ - Class Assays: ‘Amphetamines’, ‘Benzodiazepines’, ‘Opiates’ assays
- No specific identification of drug(s) in positive urine
  - Combinations below give same ‘opiates’ positive result:

    morphine

    morphine + hydromorphone

    morphine + hydrocodone + hydromorphone

Specific identification requires confirmation testing

## 4. Assay Immunoreactivity For A Drug Determines Drug Detection Limit

- Class assay has varying reactivities to members of the ‘class’:
  - Opiates assay: morphine > hydrocodone > hydromorphone > oxycodone
  - Benzodiazepines assay: oxazepam > alprazolam > clonazepam

# Opiates Relative Reactivities

(Assay at University of Rochester Medical Center)

| **Opiate** | **Conc Equivalent to 300 ng/ml Cutoff Calibrator (morphine)** |
|---|---|
| Morphine (calibrator) | 300 |
| Codeine | 150 |
| Hydrocodone* | 650 |
| Hydromorphone* | 1400 |
| Oxycodone* | 10500 |
| Oxymorphone* | 37000 |

*** False negative possible after pain-control doses**

## 5. Assay Immunoreactivities Vary With Assay Manufacturers

- Immuno-reactivities to members of the drug ‘class’ vary with assays from different manufacturers
  - Labs using different assays may get different results

# Opiates (ng/ml) giving Positive Opiates Assay at 300 ng/ml Cutoff

| | Opiates Immunoassays | | | | | |
|---|---|---|---|---|---|---|
| **Drug** | **A** | **B** | **C** | **D** | **E** | **F** |
| **Morphine** | **300** | **300** | **300** | **300** | **300** | **300** |
| **Codeine** | **180** | **200** | **300** | **180** | **224** | **204** |
| **Hydromorphone** | **557** | **900** | **500** | **4000** | **1425** | **498** |
| **Dihydrocodeine** | **283** | **600** | **300** | **450** | **510** | **291** |
| **Hydrocodone** | **190** | **1000** | **300** | **1700** | **1086** | **247** |
| **Oxycodone** | **2644** | **1700** | **20000** | **16000** | **>75000** | **2550** |
| **Oxymorphone** | **2000** | **Unreactive** | **40000** | **----** | **40000** | **>20000** |

Modified from Krasowski, http://www.biomedcentral.com/1471-227X/9/5

## 6. Assay Cutoffs

Some immunoassays are FDA-cleared for two cutoffs

- Amphetamines: 1000 or 500 ng/ml
- Benzodiazepines: 300 or 200 ng/ml
- Cannabinoids: 50 or 20
- Cocaine: 300 or 150 ng/ml (benzoylecgonine)
- Opiates: 2000 or 300 ng/ml

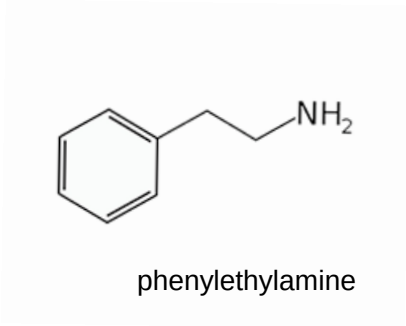
Using the low cutoff will increase detection rate

# Specific Drug Class Immunoassays

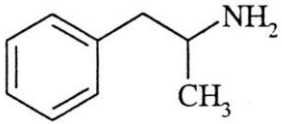
# Amphetamines

# Amphetamines

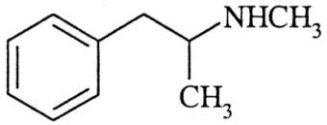
- Class of drugs sharing a common phenylethylamine structure, with varying degree of stimulant/sympathomimetic activity



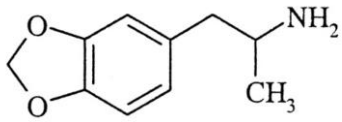

phenylethylamine



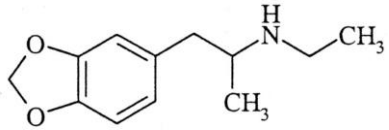

amphetamine




methamphetamine




3,4-methylenedioxyamphetamine (MDA)




3,4-methylenedioxyethamphetamine (MDEA)

# Amphetamines

Amphetamine, methamphetamine:

- Potent central nervous system (CNS) stimulants
- Enantiomers:
  - *d*-methamphetamine/*d*-amphetamine are pharmaceuticals
  - *l*-methamphetamine has 1/10 CNS stimulant effect of *d*-methamphetamine, but has greater peripheral vasoconstrictive effect (over-the-counter nasal inhaler)
  - Illicit methamphetamine is *d,l*-racemic mixture

# Amphetamines Immunoassays

- Amphetamines immunoassays target *d*-methamphetamine/*d*-amphetamine; have varying ability to detect
  - *l*-isomers
  - MDMA/MDA and the sympathomimetic amines
- False positives:
  - Bupropion, labetalol, trazadone, phenothiazines, many others
  - Eliminated by confirmation test

## Patients On Methamphetamine Or Amphetamine-containing Medications:

- Will be positive by both screen and confirmation tests:
  - Screen test (amphetamines immunoassay) will be positive
  - Confirmation test will show methamphetamine and/or amphetamine positive
- Result is an <u>ANALYTICAL</u> <u>TRUE</u> positive, but is a <u>CLINICAL</u> <u>FALSE</u> positive (for suspected illicit drug use)
- Need to know medications that contain methamphetamine or amphetamine

## Examples of Amphetamines-Containing Products

| | Amphetemines-containing Products |
|---|---|
| Substances known to contain *d*-amphetamine or *d, l*-amphetamine | Adderall<br>Dexedrine<br>Dextrostat |
| Substance known to contain *d*-methamphetamine | Desoxyn |
| Substances known to contain *l*-methamphetamine | Vicks Inhaler |
| Substances known to metabolize to methamphetamine (and amphetamine) | Benzphetamine (Didrex)<br>Dimethylamphetamine<br>Famprofazone<br>Furfenorex<br>Selegiline (Eldepryl) |
| Substances known to metabolize to amphetamine | Amphetaminil<br>Clobenzorex<br>Ethylamphetamine<br>Fenethylline<br>Fenproporex<br>Mefenorex |

Kwong, in Handbook of Drug Monitoring Methods. Humana Press 2008

# Benzodiazepines

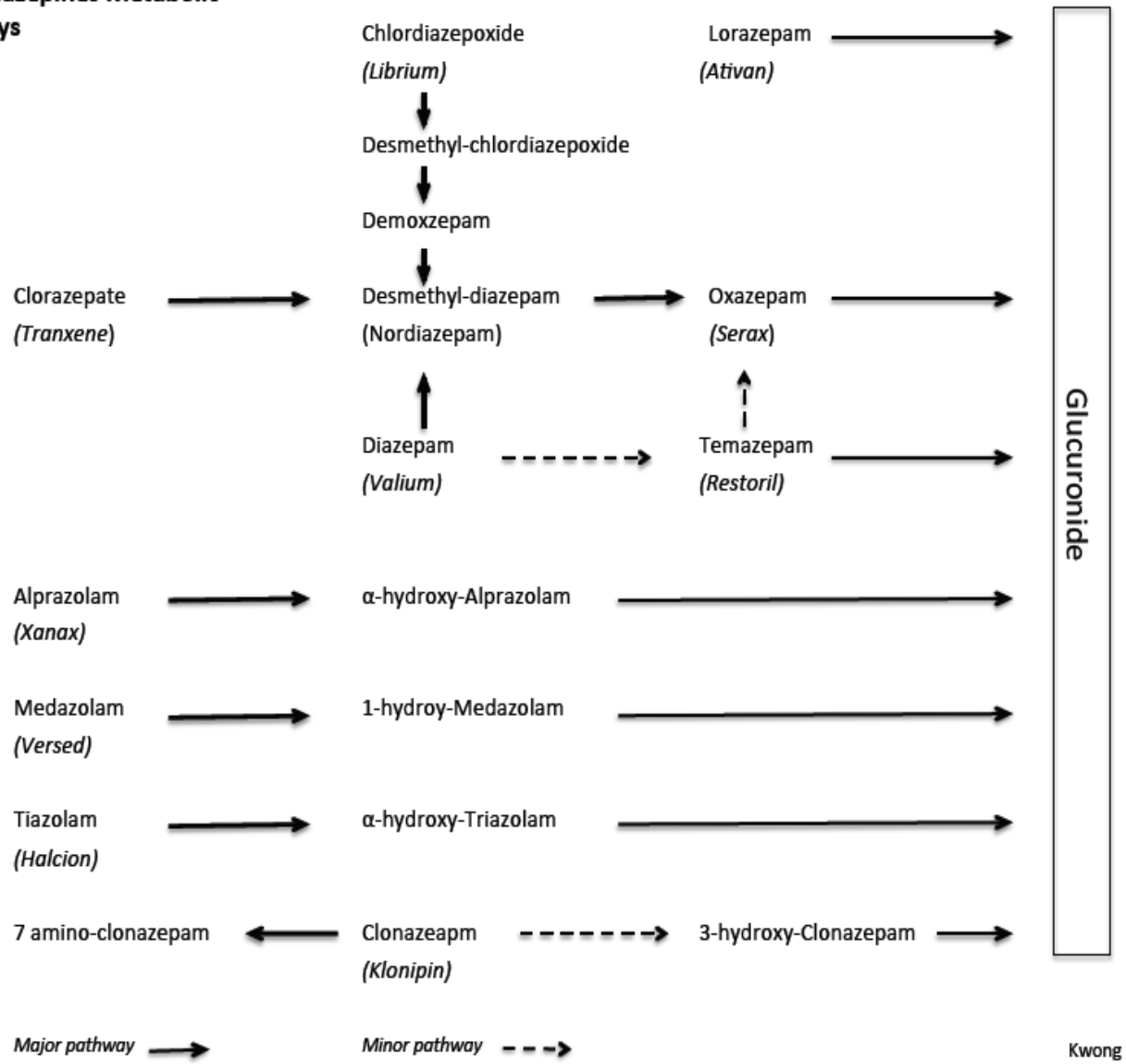
# Benzodiazepines Immunoassays Cross-reactivities

**Table 26-4 Cross-reactivity[a] (%) of Several Benzodiazepine Immunoassays at Cutoff[b]**

| Benzodiazepine | Abbott architect | Biosite triage | Microgenics CEDIA | Roche cobas | Siemens EMIT |
|---|---|---|---|---|---|
| Alprazolam | 190 | 67 | 205 | 91 | 308 |
| α-Hydroxyalprazolam | NA | 75 | 193 | 54 | 200 |
| Clonazepam | 40 | 86 | 140 | 65 | 34 |
| 7-Aminoclonazepam | 8 | NA | NA | 69 | 9 |
| Diazepam | 211 | 86 | 247 | 93 | 286 |
| Flunitrazepam | 114 | 86 | 135 | 71 | 143 |
| Lorazepam | 20 | 55 | 122 | 59 | 33 |
| Lorazepam glucuronide | NA | 75 | 1 | NA | NA |
| Nordiazepam | 200 | 100 | 211 | NA | 182 |
| Oxazepam | 100 | 43 | 107 | 77 | 80 |
| Temazepam | 160 | 55 | 144 | 78 | 143 |
| Triazolam | 160 | 75 | 191 | 85 | 154 |

Kwong, in Clinical Toxicology Laboratory, AACC Press 2013

**Benzodiazepines Metabolic Pathways**




Kwong 2013

# Benzodiazepines Immunoassays

- Most assays target either oxazepam or nordiazepam
  - May detect poorly those drugs not metabolized to oxazepam or nordiazepam:
  - Most prescribed benzodiazepines are not detected well: alprazolam (46$^{th}$ most prescribed 2010), clonazepam (47$^{th}$), and lorazepam (78$^{th}$) - **False negative possible**
  - Newer drugs for insomnia: zolpidem (Ambien®) and eszoplicone (Lunesta®) are not detected

# Opiates

# Opiates Immunoassays

**‘Standard’** Opiate immunoassays

- Target morphine
- Lesser reactivity with hydromorphone, hydrocodone, dihydrocodeine, oxycodone, oxymorphone
- **False negative possible for the semi-synthetics after pain-control doses**

# Opiates Immunoassays

**'Standard'** Opiate immunoassays

- True positive: poppy seeds contaminated with morphine
- False positives: ofloxacin, levofloxacin
- No reactivity with non-opiate opioids: methadone, buprenorphine, fentanyl, tramadol, meperidine (separate assays available)

# Buprenorphine Immunoassay Cross-Reactivities (5 ng/ml cutoff)

| Substance | % Cross Reactivity | Conc ng/ml Equivalent to 5 ng/ml Buprenorphine |
|---|---|---|
| Morphine | 0.010 | 50,0000 |
| Codeine | 0.016 | 31,250 |
| Dihydrocodeine | 0.014 | 35,714 |
| Hydrocodone | 0.013 | 38,462 |

Modified from Bottcher, J Analyt Tox, 2005

# Thank you for attending!

**Please join me in the Networking Lounge for an online Q&A session.**

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## Self-Assessment Questions

1. Which of the following statements on immunoassays is INCORRECT?

    a. All FDA-cleared opiates immunoassays use morphine as the calibrator drug

    b. Immunoassays used in urine drug testing are qualitative assays

    c. A test is positive if the test sample reaction absorbance is equal to that of the calibrator at cutoff concentration

    d. A negative test result indicates the absence of drug in the urine

## Self-Assessment Questions

2. Which of the following statements on immunoassays is INCORRECT?
   a. Immunoassay results are typically reported as positive or negative
   b. An immunoassay positive result is a presumptive positive result because it can be a false positive or true positive
   c. An opiates positive result after the consumption of poppy seeds is an analytical false positive
   d. A drug that has lower reactivity relative to that of the calibrator drug has a higher detection limit
   e. Assays from different manufacturers can have different detection limits for the same drug

## Self-Assessment Questions

3. Which of the following statements on opiates immunoassays is INCORRECT?

   a. A urine containing morphine or morphine plus hydrocodone will give the same opiates immunoassay positive result

   b. A patient on buprenorphine will have a negative opiates test because buprenorphine is not an opiate

   c. Using the 2000 ng/ml cutoff instead of the 300 ng/ml cutoff can eliminate a majority of positive results due to poppy seeds consumption

   d. Using the 2,000 ng/ml cutoff will increase the detection rate for hydromorphone

## Self-Assessment Questions

4. Which of the following drugs is NOT an opiate
    a. Oxycodone
    b. Methadone
    c. Hydrocodone
    d. Hydromorphone

## Self-Assessment Questions

5. Which of the following statements on urine drug testing is INCORRECT?

   a. Many of the benzodiazepines immunoassays detect poorly the use of clonazepam

   b. Detection of lorazepam use can be enhanced by a hydrolysis step prior to the immunoassay reaction because the assay antibody poorly detects the conjugate metabolite

   *c.* *d*-methamphetamine and *l*-methamphetamine are strong central nervous system stimulants

   d. Some of the available amphetamines immunoassays can be used clinically for detection of 3,4-Methylenedioxymethamphetamine (MDMA)