Analytical Methods Used in Urine Drug Monitoring (UDM)

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Analysis of Urine Opioids/Opiates

• What & How

• Problems

• Recent developments

• Operational realities
Naturally Occurring

- Morphine
- Papaverine
- Noscapine
- Thebaine
- Codeine
Semisynthetic

Heroin (6-MAM)
Hydrocodone
Hydromorphone
Oxycodone

Oxymorphine
Buprenorphine
Dihydrocodeine
Acetylcodine
<table>
<thead>
<tr>
<th>Synthetic</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Methadone</td>
<td>Pentazocine</td>
</tr>
<tr>
<td>Fentanyl (family)</td>
<td>Levorphanol</td>
</tr>
<tr>
<td>Tramadol</td>
<td>Butorphanol</td>
</tr>
<tr>
<td>Meperidine</td>
<td>Nalbuphine</td>
</tr>
<tr>
<td>Propoxyphene</td>
<td>Nalorphine</td>
</tr>
</tbody>
</table>
Screening Methods - Immunoassay

Formats
- RIA (uncommon)
- EIA (liquid reagent)
- ELISA (96 well plate)
- Immunochromatography (POC)

Vendor specific detection techniques
- EMIT
- FPIA
- KIMS
- CEDIA

Diagram:
- Tracer (labeled drug)
- Antibody (competitive binding)
- Antigen (drug)
Generalizations

**Advantages**
- Low labor cost (easy to automate)
- Low to moderate reagent cost
- Low tech
- Rapid (POC)
- Good sensitivity

**Disadvantages**

**Specificity**
- Reacts with unwanted compounds = False POS
- Doesn’t react with all compounds in drug class = False NEG
- Cross-reactivity can vary with
  - Format
  - Vendor
  - Reagent Lot
## “Other” Immunoassays

<table>
<thead>
<tr>
<th>Drug/Class</th>
<th>Notable Feature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamines</td>
<td>false positives</td>
</tr>
<tr>
<td>Barbiturates</td>
<td>low incidence pos</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>false negatives</td>
</tr>
<tr>
<td>Cannabinoids (THC)</td>
<td>specific</td>
</tr>
<tr>
<td>Cocaine metab (BE)</td>
<td>highly specific</td>
</tr>
<tr>
<td>Phencyclidine (PCP)</td>
<td>false positives</td>
</tr>
</tbody>
</table>
Opiate Class Immunoassays

- Excellent for morphine & codeine
- Variable detection: semisynthetics
- False negatives: synthetics & semisynthetics
- Two common cutoffs—Use 300, not 2000 ng/mL
“The following compounds tested POSITIVE on the Brand X Opiate assay at the 300 ng/mL cutoff”

<table>
<thead>
<tr>
<th>Drug</th>
<th>ng/mL (%)</th>
<th>Drug</th>
<th>ng/mL (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dihydrocodeine</td>
<td>400 (75)</td>
<td>Morphine-3-G</td>
<td>375 (80)</td>
</tr>
<tr>
<td>Hydrocodone</td>
<td>350 (86)</td>
<td>Naloxone</td>
<td>6,000 (5)</td>
</tr>
<tr>
<td>Hydromorphone</td>
<td>350 (86)</td>
<td>Naltrexone HCl</td>
<td>50,000 (&lt;1)</td>
</tr>
<tr>
<td>Imipramine</td>
<td>20,000 (2)</td>
<td>Ofloxacin</td>
<td>100,000 (1)</td>
</tr>
<tr>
<td>Levorphanol tartrate</td>
<td>100,000 (&lt;1)</td>
<td>Oxycodone</td>
<td>10,000 (3)</td>
</tr>
<tr>
<td>Meperidine</td>
<td>150,000 (&lt;1)</td>
<td>Oxymorphone</td>
<td>20,000 (2)</td>
</tr>
</tbody>
</table>

Example of package insert information from POC and Laboratory Instrument Immunoassay kits for abused drugs in urine.
SFGH - Opiate Immunoassay Dose-Response Curves June 2007

Amount of Drug Added to Drug Free Urine (ng/ml) vs. Assay Response

- X = Y
- 300 ng/mL Assay Cutoff
- 300 ng/mL concentration
SFGH - Opiate Immunoassay Dose-Response Curves June 2007

- **Codeine**
- **Morphine**
- **X=Y**

**Assay Cutoff**
- 300 ng/mL

**300 ng/mL concentration**

Amount of Drug Added to Drug Free Urine (ng/ml) vs. Assay Response

- **X = Y**
- **300 ng/mL Assay Cutoff**
- **300 ng/mL concentration**
86% cross reactivity

Hydromorphone

Hydrocodone

X=Y

"Positive at 350 ng/mL"

"86% cross-reactivity"

"~50% cross-reactivity"
Opiate Immunoassay Dose-Response Curves

- Oxycodone
- Oxymorphone
- \( X=Y \)

300 ng/mL concentration

300 ng/mL Assay Cutoff

“Positive at 10,000 ng/mL”

“Positive at 20,000 ng/mL”
Opiate Class Immunoassay
Detection of Semisynthetic Opioids

• Cross-reactivity studies are not straightforward AND verification by users is not regulated

• How best to convey the information?

• They do not answer the question – if my patient takes “X” dose(s) at “Y” time - should the test be positive?
Other Opioid Immunoassays

• Methadone – why not instead use ……..

• EDDP (methadone metabolite)

• Oxycodone – cross-reactivity with metabolites?
Oxycodone Compliance?

- Oxycodone Immunoassay Cutoff = 100 ng/mL
- Patient Specimen = “130”
- GCMS confirmation:
  - Oxycodone = <10 ng/mL
  - Oxymorphone = 80 ng/mL

w/o Oxymorphone detection in Immunoassay and in GCMS assay

\[ \text{False Negative} \]
Other Opioid Immunoassays (cont.)

- Propoxyphene - less used

- Buprenorphine
  - cross reactivity with morphine & codeine?
  - cross reactivity with metabolites?
Comparing Two BUPRENORPHINE Immunoassays

Opiate Cross-Reactivity

- Morphine
- Codeine
- Hydromorphone
- Hydrocodone

Buprenorphone Metabolite Cross-Reactivity

- BUPG
- NBUP
- NBUPG

BupG

Norbup

Concentration (ng/mL)
Patient Urine Opiate Concentrations

• Specimen X
  
  Codeine = 540,000 ng/mL  
  Morphine = 39,000 ng/mL

• Specimen Y
  
  Morphine = 290,000 ng/mL  
  Hydrocodone = 14,900 ng/mL  
  Hydromorphone = 3,800 ng/mL
Effects of Opiate Cross-reactivity on Buprenorphine Prevalence Study
(10 ng/mL cutoff)

Summary of Buprenorphine Immunoassay Study

<table>
<thead>
<tr>
<th></th>
<th>number</th>
<th>% of total</th>
<th>BUP average</th>
<th>average dose</th>
<th>average opiate value</th>
</tr>
</thead>
<tbody>
<tr>
<td>total tests</td>
<td>871*</td>
<td>NA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total positive</td>
<td>101</td>
<td>11.6</td>
<td>72.8 ± 34.8</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>true positives</td>
<td>83</td>
<td>9.5</td>
<td>84.6 ± 26.1</td>
<td>17mg/day</td>
<td></td>
</tr>
<tr>
<td>false positives</td>
<td>18</td>
<td>2.1</td>
<td>18.9 ± 8.3</td>
<td>1.1 ± 1.9</td>
<td>22949</td>
</tr>
</tbody>
</table>

*average age 45, 35% female, 65% male

immunoassay results = Diversion !! (2.1% of all urines tested in 30 days)

immunoassay results = all positives have a buprenorphine scrip
Effects of Buprenorphine Metabolite Cross-reactivity on Compliance Monitoring

10 ng/mL Cutoff

<table>
<thead>
<tr>
<th>Result</th>
<th>Result</th>
<th># days missed</th>
</tr>
</thead>
<tbody>
<tr>
<td>POS (84.6)</td>
<td>POS (86.9)</td>
<td>0</td>
</tr>
<tr>
<td>POS (56.7)</td>
<td>POS (91.8)</td>
<td>1</td>
</tr>
<tr>
<td>POS (32.6)</td>
<td>POS (67.8)</td>
<td>4</td>
</tr>
<tr>
<td>POS (20.6)</td>
<td>POS (50.2)</td>
<td>5</td>
</tr>
<tr>
<td>NEG (4.0)</td>
<td>POS (27.0)</td>
<td>14</td>
</tr>
<tr>
<td>NEG (4.2)</td>
<td>POS (12.2)</td>
<td>13</td>
</tr>
</tbody>
</table>

Plasma Concentration (ng/mL)
Analytical techniques – GCMS & LC-MSMS

- Chromatographic separation by GC or LC
- GC separates “better”
- LC more “compatible” with polar compounds (e.g. metabolites)

- Followed by a second “separation” using single stage or dual stage mass analysis
**Hydrocodone** (LC-MSMS product ion spectrum)
Codeine (LC-MSMS product ion spectrum)
More is better with mass analysis

• Gold standard has been GCMS (gas chromatography with single stage mass analysis – “single quadrupole”)

• Rapidly changing to LC-MSMS (liquid chromatography with dual stage mass analysis – “triple quadrupole”)

• LC-MSMS -- Faster, Better, Cheaper?
GCMS Single Quadrupole

Oxycodone

Abundance

m/z →

IONs

source slit

quadrupole rods

resonant ion (detected)

non-resonance ion (not detected)

exit slit (to detector)

TO DETECTOR

70, 112, 140.1, 173.1, 2011, 230, 258.1, 315.1
LC-MSMS - Triple Quad
MRM – Multiple reaction Monitoring (MSMS)

1. Precursor ions selection in Q1-No Isolation time.
2. Fragmentation in Q2-for a richer fragmentation pattern, no low mass cutoff
### Generalizations – Mass Spectrometry

#### Advantages
- Excellent Specificity
- Accurate quantitation
- Ability to identify many metabolites and parent drugs in one run (LC-MSMS)
- Capable of lower detection than immunoassay (LC-MSMS)
- LC-MSMS less sample prep than GCMS = cheaper/faster

#### Disadvantages
- High instrument cost ($200,000 – $400,000 for LC-MSMS)
- Highly complex – skilled labor = high labor expense
- Low to high reagent costs
- No POC – less rapid TAT
- Training/Education/Standards/Guidelines needed
Opioid Metabolite Analysis using MS

- Glucuronidates not compatible with GCMS

- The method for removing glucuronidates (hydrolysis) is often optimized ONLY for morphine-3-glucuronide.

- But all opioid glucuronidates are not the same……..

Incomplete recovery of prescription opioids in urine using enzymatic hydrolysis of glucuronide metabolites.
GCMS of Opioids in urine – missing glucuronides?

<table>
<thead>
<tr>
<th>Opioid</th>
<th>Drug Concentrations in Patient Urines: Enzymatic vs Acid Hydrolysis (acid = 100%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (ng/mL)</td>
</tr>
<tr>
<td>Codeine</td>
<td>21% (e.g. 84 vs 400)</td>
</tr>
<tr>
<td>Morphine</td>
<td>64% (e.g. 256 vs 400)</td>
</tr>
<tr>
<td>Hydromorphone</td>
<td>54% (e.g. 216 vs 400)</td>
</tr>
<tr>
<td>Oxycodone</td>
<td>99% (e.g. 396 vs 400)</td>
</tr>
<tr>
<td>Oxymorphone</td>
<td>62% (e.g. 248 vs 400)</td>
</tr>
</tbody>
</table>
LC-MSMS of Opioids in urine

• Glucuronides can be measured directly – powerful new technique………..

• But a key LC-MSMS limitation is negative interference

• Negative interferences usually more difficult to identify than positive interference
Never forget “Operational” errors

- Urine cup labeling error (pre-analytical)
- Transfer from urine cup to tube labeled for a different patient (aliquot error)
- Instrument samples air bubble or short samples (analytical error)
- Results entered for wrong patient (post-analytical error)
- Wrong specimen sent out for confirmation
San Francisco General Hospital

Then........

and Now........
UCSF at SFGH Toxicology

Katherine Chen, Supervisor
Susan Gross, Senior Supervisor

Art Epstein, Debbie Kane, Joie Meiko, Marilyn Weeks
Eva Wong – CLSs

Ping Wang, Julia Drees and Kara Lynch – Clinical Chemistry fellows

Alan Wu, Director