The Nitty-Gritty of Drug Testing

Justin Lockwood
Assistant Director
Western Regional Day Report Center
Cabell/Wayne County, West Virginia

Lauren Richards Waugh, Ph.D.
Assistant Professor
Marshall University Forensic Science Graduate Program
The **iCup 13 Panel Drug test** for the following drugs:

THC (marijuana, pot, grass, weed, hash, Mary Jane, dope)
Cocaine (coke, crack, blow, nose candy, snowball, tornado)
Opiates (heroin, morphine, opium, smack, thunder, hell dust)
Amphetamines (Dexedrine, speed, uppers)
Methamphetamines (meth, crank, ice, chalk, black beauties, crystal meth)
Buprenorphine (Suboxone, Subutex, Temgesic)
Methadone (Dolophine, Methadose, Physetone)
Oxycodone (Percocet, Percodan, Oxycontin, Tylox, oc, ox, oxy)
Barbiturates (barbs, downers, Amytal, Nembutal, Seconal, Tuinal)
Benzodiazepines (Valium, Xanax, Librium, Ativan, Halcion, Diazepam)
Propoxyphene (Darvocet, Darvon)
Phencyclidine (PCP, angel dust)
Tricyclic Antidepressants (Nortriptyline)
<table>
<thead>
<tr>
<th>Drug</th>
<th>Detection Period</th>
<th>Cutoff Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocaine COC</td>
<td>2-4 Days</td>
<td>300 ng/mL</td>
</tr>
<tr>
<td>Marijuana THC</td>
<td>15-30 Days</td>
<td>50 ng/mL</td>
</tr>
<tr>
<td>Opiates OPI</td>
<td>2-4 Days</td>
<td>2000 ng/mL</td>
</tr>
<tr>
<td>Amphetamines AMP</td>
<td>2-4 Days</td>
<td>1000 ng/mL</td>
</tr>
<tr>
<td>Methamphetamine mAMP</td>
<td>3-5 Days</td>
<td>1000 ng/mL</td>
</tr>
<tr>
<td>Phencyclidine PCP</td>
<td>7-14 Days</td>
<td>25 ng/mL</td>
</tr>
<tr>
<td>Benzodiazepines BZO</td>
<td>3-7 Days</td>
<td>300 ng/mL</td>
</tr>
<tr>
<td>Barbiturates BAR</td>
<td>4-7 Days</td>
<td>300 ng/mL</td>
</tr>
<tr>
<td>Methadone MTD</td>
<td>3-5 Days</td>
<td>300 ng/mL</td>
</tr>
<tr>
<td>Tricyclic Antidepressants TCA</td>
<td></td>
<td>1,000 ng/mL</td>
</tr>
<tr>
<td>Oxycodone</td>
<td>2-4 Days</td>
<td>100 ng/mL</td>
</tr>
<tr>
<td>Propoxyphene</td>
<td>1-2 Days</td>
<td>300 ng/mL</td>
</tr>
<tr>
<td>Buprenorphine BUP (suboxone, subutex)</td>
<td>2-3 Days</td>
<td>10 ng/mL</td>
</tr>
</tbody>
</table>
Substances Used as Adulterants

- Alcohol
- Ammonia
- Bleach
- Ascorbic acid
- Bleach
- Blood
- Detergent
- Drano
- Golden Seal Root
- Lemon Juice
- Lime-A-Way
- Peroxide
- Salt
- Soap
- Sodium Phosphate
- Vanish
- Vinegar
- Visine
Diluents

- *in vivo* – ingestion of diuretics, drink copious amounts of water, eat or drink certain foodstuffs

- *in vitro* – add liquid to the sample
Methods of adulterations
- **Top – left**
  - Soapy urine

- **Top – right**
  - Urine with Drano

- **Bottom**
  - Key for adulteration test strip
Urine Collection Procedure
Step One:

Client empties pockets
Step Two:

Employee prepares using protective gloves
Step Three:

Client washes hands
Step Four:

Collector puts on rubber gloves
Step Five:

Client passes instant screen
Step Six:

Client provides sample
Step Seven:

Provide sample, read results
Step Eight:
Client admits or denies test results
Step Nine:
Results are scanned, faxed
Questions for Discussion?
Benefits and Limitations of Testing Methodologies and Samples
Testing Conditions

- All individuals must be informed they are subject to testing
- Confidentiality of the test results must be assured
- All positive results on the initial screen must be confirmed with alternate methodology
- Random screening for drug use under a well-defined program is appropriate and legally defensible in certain circumstances
Screening and Confirmation

Forensic Scientists determine the identity of drugs using...

- **Screening Tests** – preliminary test used to reduce the number of possible identities of an unknown substance
  - Immunoassay

- **Confirmation** – a single test that specifically identifies a substance
  - GC/MS
What is an Immunoassay?

- Method of screening samples
- Developed to target certain drug(s) within each class
- Antibody – Antigen reaction
- Antibody specific for drug and/or metabolites
- Competitive reaction between drug in collected sample and a drug with a label added to the reaction
General Immunoassay Information

- Drug(s) do not need to be extracted from the sample
  - Minimal sample preparation
- Results are semi-quantitative
- May react with multiple drugs within a class of drugs
- May be sensitive to interferences added to the sample (sample adulteration)
Parts of an immunoassay for Oxycodone

Antibodies that recognize Oxycodone

Oxycodone molecules bound to an enzyme that makes a color change occur
Molecules of Oxycodone in Urine to be sampled
Add a small sample of urine to the immuno-assay.
Incubate sample for a bit.
Added to the mixture…

= Molecule that “fits” into enzyme

The enzyme makes a colored molecule that we can see

Will be taken up by the enzyme
Color change cannot happen if enzyme is blocked by antibody
Lots of Oxycodone in Urine....
Not Much Oxycodone in Urine....
## Target Analytes

<table>
<thead>
<tr>
<th>Drug/Drug Class</th>
<th>Target Analyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamines*</td>
<td>Amphetamine/Methamp.</td>
</tr>
<tr>
<td>Cannabinoids*</td>
<td>Carboxy-THC</td>
</tr>
<tr>
<td>Cocaine*</td>
<td>Benzoyllecgonine</td>
</tr>
<tr>
<td>Opiates*</td>
<td>Morphine</td>
</tr>
<tr>
<td>Phencyclidine (PCP)*</td>
<td>PCP</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>Nordiazepam/oxazepam</td>
</tr>
<tr>
<td>Barbiturates</td>
<td>Secobarbital</td>
</tr>
<tr>
<td>LSD</td>
<td>LSD</td>
</tr>
<tr>
<td>Methadone</td>
<td>Methadone</td>
</tr>
<tr>
<td>Propoxyphene</td>
<td>Propoxyphene</td>
</tr>
</tbody>
</table>

* represents drugs that are mandated by the Federal Government for "Sensitive Occupations". Referred to as "NIDA 5" or "DHHS 5".
Immunoassays Can Work for a Class of Drugs

Structure 1. Structures of selected benzodiazepines.
Positive Immunoassay Results Must be Confirmed

- Cut-off levels differ between DHHS and DOD

<table>
<thead>
<tr>
<th></th>
<th>HHS</th>
<th>DoD</th>
<th>(ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamines</td>
<td>1000</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>Barbiturates</td>
<td>*</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Cannabinoids</td>
<td>50</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Cocaine metabolite</td>
<td>300</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td>PCP</td>
<td>25</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Opiates</td>
<td>300</td>
<td>2000</td>
<td></td>
</tr>
<tr>
<td>LSD</td>
<td>*</td>
<td>0.5</td>
<td></td>
</tr>
</tbody>
</table>

* Drug class not included in this program

- Confirmation of Positives (above cut-off)
  - Isolation of drugs/metabolites by extraction
  - Identification: specific drug of abuse
  - Quantitation: amount detected
A typical immunoassay instrument and results.
Confirmation by GC-MS

- GC - Gas chromatography
- MS - Mass spectrometry
Chromatography

- An analytical technique used to separate the components of a mixture for the purpose of identification and quantification.
- Useful to forensic scientists because drugs often come in the form of a mixture.
What a GC Looks Like Inside

- Separates molecules based on
  - Size
  - Chemical properties
- Information provided
  - Retention time – how long the drug molecules stay in the instrument
What GC Data looks like
Mass Spectrometry

- Often used as a detector for Gas Chromatography
- A substance is bombarded with high-energy electrons
- The molecule picks up a positive charge and then breaks apart into fragments
- The fragments are separated by mass
- The fragmentation pattern is unique to each individual substance
Mass spectrum of Heroin

- Each line on the graph represents pieces of the heroin molecules that came out of the GC.
- What forensic scientists look for:
  - Are all the correct pieces present?
  - Are they there in the relative amounts expected?
Mass Spectra of a few Drugs of Abuse

- 6-monoacetyl-morphine
- Oxycodone
- Cocaine
Samples used for Forensic Drug Testing
Urine

- Specimen of choice for workplace drug testing
- Urine is composed of blood filtered by the kidneys and presents a much cleaner sample than blood (fewer endogenous compounds to interfere with detection)
- Drug concentrations are almost always higher in urine than in blood
- Some drugs remain in urine for days (or longer) after use
- Concentrations vary depending on many factors
Urine

- Helpful in finding drugs that are difficult to detect in blood
- Drug quantitations in urine are almost always meaningless
- Urine drug concentrations should **NOT** be used to imply a level of impairment
- The presence of a drug in the urine indicates what drug(s) to look for and quantitate in the blood
Factors Complicating Urine Drug Concentration Interpretation

- Urine flow
- Urine pH
- Time since last bladder evacuation
- Volume of fluid intake
- Dose and time since ingestion
- Single dose vs. multiple dose
- Kidney function
- Liver function
Detection Times of Common Drugs of Abuse in Urine

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Duration of Detection in Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol</td>
<td>up to 1 day</td>
</tr>
<tr>
<td>Amphetamines (including MDMA, MDA)</td>
<td>1-2 days</td>
</tr>
<tr>
<td>Barbiturates</td>
<td>1-3 days</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>Up to 21 days</td>
</tr>
<tr>
<td>Cannabinoids</td>
<td>Up to 60 days*</td>
</tr>
<tr>
<td>Cocaine</td>
<td>1-3 days</td>
</tr>
<tr>
<td>Methadone</td>
<td>1-3 days</td>
</tr>
<tr>
<td>Opiates (including codeine and morphine)</td>
<td>1-3 days</td>
</tr>
<tr>
<td>Propoxyphene</td>
<td>1-3 days</td>
</tr>
</tbody>
</table>

*At 50 ng/mL cutoff concentration
Positive Result Interpretation

- Usually reads “Unable to detect” or “None detected”
- Drug or drug class indicated is present in the urine
- Person is not necessarily under the influence at the time of collection
- Some time frame of use can possibly be provided
- Single positive result provides limited information
Negative Result Interpretation

- Subject is not using a drug detected by test
- Taking a drug detected, but
  - Dose too small to detect
  - Not taking frequently enough to detect
  - Urine collected too long after ingestion
  - Sample was adulterated
- Sensitivity of the assay was not sufficient
Summary:

- Testing involves a screening step and the confirmation of the positive result.
- Urine is the most frequently used sample, but oral fluid, sweat, and hair have also been used.
- Samples can be adulterated with mixed results for success.
- Child welfare agencies and substance abuse agencies can develop comprehensive policies and procedures to design and implement successful testing.
QUESTIONS???