Toxicology 101

Arizona Problem Solving Courts
April 25, 2016

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Toxicology is defined as the study of the adverse effects of chemicals on living organisms.

Forensic toxicology is the use of toxicology along with analytical chemistry, pharmacology and clinical chemistry to aid medical or legal investigation of death, poisoning, and drug use.
Forensic Toxicology Process

Collections
Chain of Custody
Accessioning
Screening tests
Validity tests
Confirmation tests
Common Analytes
Alcohol vs EtG/EtS
Synthetics/Designer drugs
Forensic Toxicology Process

Collections
Chain of Custody
Accessioning

Screening tests
Validity tests
Confirmation tests
Common Analytes
Alcohol vs EtG/EtS
Synthetics/Designer drugs
Urine

- Most common matrix
- Need to be looking for correct analyte (parent vs. metabolite)
- Very dependent upon metabolism and detection windows
- High concentration of analytes
- Relatively inexpensive
- Large sample volume
- Cannot be related to impairment
- Short term use (1-7 days)
- Collection difficulties/regulations
Types of Samples

Hair

- Good for long term, periodic testing.
- Difficult interpretation
- Susceptible to environmental contamination
- Difficult sample preparation
- Expensive
- Non-invasive collection
- No correlation to impairment
- Sample availability
Oral Fluid

- Non-invasive collections
- Lower concentrations of analytes
- Limited specimen volume and stability
- Short detection windows
- Possible correlations to dosage and effects
- Some analytes not present
- Possibility of environmental contamination
- Relatively expensive
- Not the same amount of research as other matrices
Screening Tests

Screening Methods

- EIA
  - EMIT (enzyme multiplied immunoassay technique)
  - ELISA (enzyme linked immunosorbent assay)

- Mass spectrometry screens
  - Relatively new
  - Often used for synthetics or complicated matrices
Screening Tests

Sensitivity vs. Specificity

➢ Sensitive but not specific.
➢ Lots of possibilities for false positive results.

Parent drugs vs. Metabolites

➢ Often cannot distinguish between the two.

Drug Families

➢ Usually detected as a whole.
➢ Cross-reactivity between similar families often happens.
Screening Tests

Good for

- Eliminating negative samples
- Treatment program compliance
- Deciding which confirmations to order

Not good for

- Legally defensible results
- Interpretation
- New use monitoring
Why use screening tests?

- High through put (1,200 tests/hour)
- Quicker turn around time
- Relatively inexpensive
- Elimination of negatives
- Confirmation decisions
- More accurate than FTKs less accurate than confirmation.
Specimen Validity Testing

Validity tests are designed to determine if a sample is consistent with unadulterated urine collected from a healthy human donor.

Types of validity tests

- General observations
- Creatinine
- Specific gravity
- pH
- Oxidants
- Soap
Specimen Validity Testing

General Observations

- Color
- Smell
- Consistency
- Particulates
- Anything that might interfere with testing
Specimen Validity Testing

Creatinine

- Skeletal muscle metabolite produced at constant rate in healthy humans
- Used as a marker for dilution
- Normal urine >20.0 mg/dL
- Dilute urine <20.0 mg/dL
- Substituted urine <2.0 mg/dL
- Not creatine
Specific Gravity

- Relative density, good indicator of adulteration
- Normal 1.0030-1.0350
- Dilute 1.0010-1.0030
- Substituted <1.0010 or >1.0200

pH

- Determines acidity or basicity of sample
- Normal urine 4.5-8.9
Specimen Validity Testing

Oxidants

- Common adulterants
- Can interfere with screening results
- Hydrogen peroxide
- Bleach
- Chromate
- Nitrite
Confirmation Testing

Tests that use a different physical-chemical property from the screening test.

Confirmation methods

- Mass Spectrometry
  - Gas chromatography (GC)
  - Liquid chromatography (LC)
  - Tandem mass spectrometry (MS/MS)

- Future Methods
  - Time of flight (TOF)
  - Ion mobility (IMS)
Chromatography

All chromatographic methods involve the partitioning of sample between a mobile phase and a stationary phase.

The interaction of a mixture with these two phases will cause it to separate out into its constituent compounds.

The two most common modern methods of chromatography are gas chromatography (GC) and liquid chromatography (LC).

Each analyte has a retention time that can be useful identification.
Chromatography
Mass Spectrometry

Method that detects analytes by their mass to charge ratio (m/z).

Can be used individually or in tandem.

Most common confirmation method.

Can be broken down into three basic steps:

➢ Ion creation
➢ Mass selection
➢ Detection
Mass Spectrometry

- Ion Source
- Second Quadrupole (MS2)
- Collision Cell
- First Quadrupole (MS1)
- ion optics
Mass Selection
Mass Spectrum
Confirmation Testings

Good for

- Legally defensible results
- Virtually no false positives
- Quantitative results
- Individual analytes
- Interpretation
- New use determination
- Therapeutic drugs
Confirmation Testings

Not good for

- General screening
- Expensive
- Time consuming
- Quick method development
- High level of expertise required
Confirmation Testings

When do I need a confirmation?

- Legal action
- Confirm all positive screening results
- Specific information
- Concerned about adulteration
- Low levels
Common Analytes

THC

Amphetamine and Methamphetamine

Cocaine

Opiates

Benzodiazepines
THC

11-nor-9-carboxy-THC (THCCOOH) common metabolite tested for in urine. Variety of other metabolites available as well.

THC is naturally lipophilic. This means that it is stored in fat inside the body. This can create a longer detection window than is seen with most analytes.

$\Delta 9$-tetrahydrocannabinol ($\Delta 9$-THC) commonly tested for in oral fluid. Active compound found in marijuana.
New use monitoring is a common goal of THC testing.

Use confirmation values, not screening values or semi-quantitative values.

New use interpretation should be done based upon a THC/Creatinine ratio. This can help account for differences in hydration between samples.

This ratio should decrease by half every 2-10 days. Dependent upon the donor’s metabolism and usage history.

The more information, the better. The presence of THC does not always mean new use.
Amphetamine/Methamphetamine

Detection window of 2-3 days.

Sympathomimetic Amines should not cause a false positive confirmation.

- Pseudoephedrine

Methamphetamine will metabolism to amphetamine.

Methamphetamine can still be present by itself.
Results are only consistent with drugs containing amphetamine if there is no methamphetamine present.

- Adderall, Benzedrine, Vyvanse, Amfetamine.

Prescriptions containing methamphetamine are extremely rare.

- Benzphetamine, Selegiline, Desoxyn.

Vicks inhaler contains levomethamphetamine. If this is being used a D vs L test can be run to determine the source of methamphetamine.
Cocaine

Several common metabolites can be tested for in urine. Norcocaine, benzoyllecgonine, ethyl cocaine.

Both parent and metabolite often tested for in oral fluid.

Detection window of 2-3 days.

Cocaine is only used medically as a topical analgesic for very specific surgeries, such as on the eye or sinuses. Even then it is only used under certain circumstances.
Common opiates
- Codeine
- Morphine
- Hydrocodone
- Hydromorphone
- Oxycodone
- Oxymorphone

Detection window of 2-3 days.

Large variety of common prescriptions available.
Complicated metabolic pathways.
6-AM

6-acetylmorphine (6AM) is a direct metabolite of heroin with a small detection window of 1 day.

6AM is useful in determining the difference between heroin and morphine use, since we cannot test for heroin directly.

Presence of 6AM is consistent with heroin use, however the absence does not rule it out completely due to short detection window.
Benzodiazepines

Common Benzodiazepines
- Oxazepam
- Nordiazepam
- Temazepam
- Lorazepam
- Alprazolam
- Flurazepam
- Midazolam
- Triazolam
- Clonazepam

Very large number of benzodiazepines with a variety of legal statuses.
**Benzodiazepines**

Benzodiazepines can be broke up into three different groups depending on the time frame they are designed to work over. Important to keep in mind due to detection windows and metabolism.

<table>
<thead>
<tr>
<th>Short (2 days)</th>
<th>Intermediate (5 days)</th>
<th>Long (10 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triazolam</td>
<td>Alprazolam</td>
<td>Diazepam</td>
</tr>
<tr>
<td>flurazepam</td>
<td>Lorazepam</td>
<td>nordiazepam</td>
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<tr>
<td></td>
<td>Oxazepam</td>
<td></td>
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<tr>
<td></td>
<td>Temazepam</td>
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<tr>
<td></td>
<td>clonazepam</td>
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</tr>
</tbody>
</table>
Benzodiazepines

Medazepam (Nobrium) (4-8 hours) → Diazepam (Valium) (3-6 days) → Temazepam (Restoril) (1-4 days)

Chlordiazepoxide (Librium) (1-4.5 days)

Chlorazepate (Tranxene) → Nordiazepam (6.5-8.5 days) → Oxazepam (1-3 days)

Halazepam

Demoxepam

Prazepam (Centrax)
12-14 hour detection window.

Fermentation can cause false positives.

Glucose being present does not always imply fermentation occurred.

Only ethanol will cause a positive result.

Urine ethanol levels do not correlate well with BAC. Not a measurement of impairment.
## Alcohol metabolism

<table>
<thead>
<tr>
<th>Method</th>
<th>Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidation-95%</td>
<td>acetaldehyde, acetate</td>
</tr>
<tr>
<td>Direct-5%</td>
<td>breath, urine, perspiration</td>
</tr>
<tr>
<td>Minor- &lt;0.1%</td>
<td>ethyl glucuronide, ethyl sulfate, ethyl phosphate</td>
</tr>
</tbody>
</table>
Traditional Biomarkers

**Indirect markers**
- Mean cell volume (MCV)
- Carbohydrate deficient transferrin (CDT)
- Liver enzymes
  - Gamma-gt
  - Alt
  - Ast

**Direct Markers**
- Blood Alcohol
- Breath Alcohol
- Urine Alcohol
Modern Biomarkers

Direct Biomarkers

Ethyl glucuronide (EtG)

Ethyl sulfate (EtS)

Ethyl phosphate (EtP)

Phosphatidylethanol (peth)
Advantages of EtG/EtS as a Biomarker

Direct biomarker of ethanol exposure

Longer detection window than alcohol (72-96 hours)

Stable (non-volatile), water soluble

Reduction of false positive confirmations

Fermentation not a factor
Disadvantages of Etg/EtS as a biomarker

Not available at all laboratories

EtG susceptible to bacterial degradation

Incidental exposure

Interpretation
Differences to Traditional Biomarkers

Not a marker for impairment

Does not correlate with BAC

Not diagnostic of alcoholism or long term use
EtG/EtS Urinalysis

Screening
Immuoassay
Semi-quantitative (100-2,000 ng/ml)
Only testing for EtG
False positives can occur
Screening False Positive Rates

EtG False Positive EIA Screening Rate January - March 2014

- n = 5,129 Confirmations
- 441 False Positives
- Overall = 8.6%
EtG/EtS Urinalysis

Confirmation
LC/MS/MS
Quantitative (50-50,000 ng/ml)
Both EtG and EtS
No false positives
Legally defensible
Incidental Exposure

Voluntary Exposure to Ethanol

Incidental Exposure to Ethanol

Not a FALSE POSITIVE Result
Incidental Exposure

Alcohol free alternatives

When in doubt don’t use, consume or apply

Use client contracts
Interpretation

How should a test cutoff value be chosen?

The cutoff value selected to distinguish produces in a category should be based on the cut-off point in the ROC curve that offers the best combination of sensitivity and specificity. The ROC curve is a plot of the true positive rate (sensitivity) against the false positive rate (1-specificity) for different cutoff points. The ROC curve is calculated by plotting the sensitivity against 1-specificity for each possible cutoff value. The area under the ROC curve (AUC) is a measure of the overall accuracy of the test. A higher AUC indicates a better test.

How can EIS and EIG best be used?

EIS and EIG can be used to assess the performance of a diagnostic test. EIS is a measure of the ability of the test to correctly identify individuals who have the disease. EIG is a measure of the ability of the test to correctly identify individuals who do not have the disease. EIS and EIG are calculated by dividing the number of true positive and true negative results by the total number of results, respectively. A higher EIS and EIG indicate a better test.

For more information, the test kit and user manual should be consulted. The test kit contains detailed instructions on how to perform the test, and the user manual contains information on how to interpret the results. It is important to follow the instructions provided in the test kit and user manual to ensure accurate results.
Recommendations

Advise clients about EtG/EtS

Educate about incidental exposure

Use client contracts

Confirm all positive screening results

Use appropriate cutoffs
Designer Drugs

Designer drugs are made by taking existing drugs and chemically modifying their structure to produce a new compound.

This allows producers to get around patents, makes detection difficult, and creates a legal grey area.

The two most common groups of designer drugs are Spice and Bath salts.
Designer Drugs - Spice

The compounds that make up the group known as Spice are synthetic cannabinoids. These are compounds that attempt to mimic the effects of THC.

Marijuana is the most commonly used illicit drug in the US, these synthetics are the second.

Previous studies have shown up to 37% of high school seniors have used marijuana, while as much as 11% have used spice.
Designer Drugs - Spice

Sold as Herbal Incense or Botanical Incense

Herbal blends with synthetic cannabinoids added.

Smoked like marijuana with similar effects

“Not for Human Consumption”
Designer Drugs - Spice

Pharmacology
- Similar to THC
- Euphoria (High)

Adverse Effects
- Psychological
  - Severe Paranoia
  - Hallucinations
  - Severe Anxiety
- Pathophysiologic
  - Elevated HR and BP
  - Seizures
  - Vomiting
  - Renal Failure
  - Death
Designer Drugs

Legal Status
Banned in all branches of military

States have banned many compounds.

Federal

Schedule 1: Controlled Substances Act

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<tbody>
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<td>XLR-11</td>
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</tbody>
</table>
Designer Drugs - Spice

Testing for Synthetic Cannabinoids
- Spice is not detected in screening or confirmatory tests for THC (marijuana)

Synthetic Cannabinoid Testing at Norchem
- Targeted Screening by LC/MS/MS
- Confirmatory Testing by LC/MS/MS
- Self-audit in order to maintain an up to date panel
Conventional bath salts contain Epsom salt or sea salts, baking soda, glycerin and some type of essential oil.

The designer drugs known as bath salts are usually part of the cathinone family.

Central nervous system stimulant with effects similar to methamphetamine.

Thought to be highly addictive.

Originally used as a research chemical.

Popular in Europe and Asia.
Designer Drugs – Bath Salts

Cathinone originates from Khat, a plant native to the horn of Africa and the Arabian peninsula.

Fresh leaves are either chewed or brewed in tea.

Causes a release of dopamine in the brain.

Sale of Khat is legal in:
- Australia
- Yemen
- Oman
- United Kingdom
Effects of Cathinones:

- Mild euphoria and excitement
- Talkative, unrealistic, emotionally unstable
- Constipation
- Anorectic
- Manic behaviors
- Drowsy hallucinations
Designer Drugs – Bath Salts

Side-effects of Cathinones:

- Elevated Heart Rate and Blood Pressure
- Anxiety and agitation
- Hallucinations
- Extreme Paranoia
- Delusions
- Seizures
- Nausea and Vomiting
- Death
Designer Drugs – Bath Salts

Analysis:

- Bath Salts are not detected in Amphetamine Screening Immunoassays.
- Immunoassays not available.
- Rapid On-Site Tests not available
- LC/MS/MS assays available for both screen and confirmation
Designer Drugs – Legal Status

Schedule 1 CSA:

- Cannabimimetic Agents
- Bath Salts
- 2-C

July 10, 2012, President Obama passed Synthetic Drug Abuse Prevention Act of 2012, which specifically identifies a number of synthetic cannabinoids and cathinones, commonly known as “spice” and “bath salts”. Use, distribution or possession is unlawful.
Designer Drugs – Future

Not going to go away.

Will most likely become a larger problem in the future.

Large demand for new products and international market.

Testing will always be behind what is available on the street.
Questions

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