Analytical and Forensic Toxicology
Jeff Lapoint, DO
Southern California Permanente Medical Group
San Diego, CA

Thanks to Drs: Brent Morgan, Evan Schwarz, Howard Greller, Jeff Brent

Content
• Core Content of Medical Toxicology
  • Part 6: Analytical and Forensic Toxicology

Scenario
• 20 yo male presents with N/V 8 hours after mushroom ingestion
  • Toxicologist has 2 plans for Amanita testing
    • Send sample to mycologist
    • Send urine sample to vet lab for α-amanitin assay
CLIA
- Clinical Laboratory Improvement Amendments of 1988
- Regulates testing of samples 'derived from human body'
- Agencies responsible
  - FDA, CMS, CDC

FDA
- Food and Drug Administration
- Categorizes tests based on complexity
- Reviews requests for Waiver by Application
- Develops rules/guidance for CLIA complexity categorization

CMS
- Center for Medicaid Services
- Issues laboratory certificates
- Collects user fees
- Inspections and enforcement of regulations
CMS continued

- Certifies private inspection organizations
- Monitors performance - Proficiency Testing (PT)
- Publishes CLIA rules and regulations

CDC

- Center for Disease Control
- Analysis, research, technical
- Manages CLIAC
  - Clinical Improvement Advisory Committee
  - Technical standards and practice guidelines

Testing Complexity

- Waived
- Moderate complexity
- High complexity

Moderate
- Record Keeping
- Written Procedures
- Laboratory Director
- Competency Testing
- Proficiency Testing
- Controls
- Inspection

High
- Qualified onset supervisor
- Daily review of all results
Limits of Testing

- Accuracy
- Precision
- Sensitivity
- Specificity

Performance Challenges

- Cutoff discrepancy
- Test not designed to detect drug

Performance Challenges

- Cutoff discrepancy
- Test not designed to detect drug
- Poor sensitivity
Performance Challenges

- Cutoff discrepancy
- Test not designed to detect drug
- Poor sensitivity
- Cross reactivity
- Calibration
- Poor specificity
- Unexpected 'false' results
- Poor alignment of confirmation test
- Poor agreement
Hair Analysis

**Strengths**
- Less invasive sampling
- Reveals exposure

**Weaknesses**
- No standards for analysis
- Contamination
- No quantitative data on exposure dose
- No population data

NIDA-5

- National Institute for Drug Abuse (NIDA)
- Recommended screening
- Federal employees and DOT occupations
  - Cannabis
  - Opiates
  - Cocaine
  - Amphetamines
  - Phencyclidine

Immonoassays

- Single drug assays (cocaine, THC) have high specificity
- Challenging to target whole classes
  - Amphetamines, BZD, opioids
  - False +: can be caused by structurally related "out of class" drugs
  - False -: antibody reacts weakly with some "in class" drugs
Federal Cutoffs (ng/mL)

<table>
<thead>
<tr>
<th>Substance</th>
<th>Screening</th>
<th>Confirmatory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocaine (met)</td>
<td>300 ng/ml</td>
<td>150 ng/ml</td>
</tr>
<tr>
<td>Opiate (met)</td>
<td>2000 ng/ml</td>
<td>2000 ng/ml</td>
</tr>
<tr>
<td>Amphetamines</td>
<td>1000 ng/ml</td>
<td>500 ng/ml</td>
</tr>
<tr>
<td>THC (met)</td>
<td>50 ng/ml</td>
<td>15 ng/ml</td>
</tr>
<tr>
<td>PCP</td>
<td>25 ng/ml</td>
<td>25 ng/ml</td>
</tr>
</tbody>
</table>

**I occasionally enjoy cocaine**

**Tetrahydrocannabinol (THC)**

- (-)-trans-Δ9-tetrahydrocannabinol
  - nordelta 9 thc or THC-COOH
  - Occasional use: 3 days
  - Chronic use: > 1 month
  - Δ9 THC whole blood 5 ng/ml
“Second hand smoking” defense
- Unlikely cause of positive screening
- Studies of passive use
  - Concentrations ~6 ng/ml
  - Heavy passive exposure 15-20 ng/ml
- NIDA cutoff 50 ng/ml

Opioids
- Morphine: Most commercial immunoassays
- Morphine: codeine, heroin metabolite
- Cross reactivity depends on assay
- Synthetics show little or no cross-reactivity
"Poppy Seed” Defense

Dextromethorphan
- Similar structure to morphine and codeine
- False + for PCP on immunoassays
- Dextrorphan – major metabolite
- Levorphanol – “L” enantiomer, also opioid
- Can’t differentiate optical enantiomers by MS
Methadone

- False Positives
  - Quetiapine
  - Doxylamine
  - Olanzapine
  - Diphenhydramine
  - Verapamil metabolites

Phencyclidine

- Immunoassay: many false positives
  - Dextromethorphan
  - Ketamine
  - Diphenhydramine
  - Buproprion
  - Venlafaxine

Benzodiazepines

- Immunoassays: oxazepam & nordiazepam
- The false-negative rate for BZD in an immunoassay screen is ~ 25-30 %
- False negatives
  - Glucuronides (lorazepam)
  - Clonazepam, Flunitrazepam, Alprazolam
Amphetamines

- Amphetamine assay plagued with false positives
  - OTC cold preps, herbs
  - Some fail to detect "designer" amphetamines

Nasal Inhalers

- Can contain l-methamphetamine
  - Isomer of d-methamphetamine
  - Both turn immunoassays positive
  - Difficult to distinguish with mass spec
  - Optical enantiomers

TCA Immunoassay

- Cross react with ringed xenobiotics
- False positives
  - Carbamazepine
  - Diphenhydramine/hydroxyzine
  - Quetiapine
  - Cyproheptadine
Adulteration

- 2004: First mandatory guidelines for federal workplace testing (SAMHSA)
- Tampering: Variable
  - In vitro adulteration
  - In vivo adulteration
- Dilution
- Urine substitution

Specimen Validity

- Appearance
- Temperature (90-100F)
- pH testing (pH 3-11)
- Specific gravity (> 1.003)
- Creatinine (> 20 ppm)

In Vitro Adulteration

“Add after urination”

- Interfere with an immunoassay or alter a target drug
- Sold under many names but contain:
  - Glutaraldehyde
  - Sodium or potassium nitrate
  - Pyridinium chlorochromate
  - Peroxide/peroxidase
In Vivo Adulteration
“Ingest prior to urination”

- Primary mechanisms: dilution and excretion
- Water and diuretics
- Fool visual inspection
- Interfere with creatinine level checks
- B-vitamins, riboflavin, creatin

Cholinesterases

- Serve as markers for poisonings
  - Butyrylcholinesterase
  - Red Cell Acetylcholinesterase

Butyrylcholinesterase

- Plasma cholinesterase (pseudo)
  - Metabolizes cocaine, succinylcholine
    - Fails first
    - Recovers first
    - Less specific
Red Cell Acetylcholinesterase

• Reflects activity at the NMJ
• Low concentrations in people

Cholinesterases

• Normal ranges vary widely between individuals and intraindividual
• Due to differences in techniques, the absolute cholinesterase values vary from lab to lab
• Even with RBC cholinesterase, the point at which toxicity begins to appear ranges from 40% to 75% of normal values.

Unmeasured cations

<table>
<thead>
<tr>
<th>Na⁺</th>
<th>HCO₃⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Anion gap (unmeasured anions)

• 95% cations measured
• 85% anions measured
• Normal gap ±/– 4

MIND THE GAP
Low Anion Gap

- Hypercalcemia
- Hypermagnesemia
- Hyperkalemia
- Lithium
- Multiple myeloma
- Hypoalbuminemia

Forensic Toxicology

Forensics

- Aid medical / legal investigation of death, poisoning, and drug use
- Concern is not legal outcome, but obtaining and interpreting results
- Chain of Custody
- List everyone who handled a specimen (special couriers)
- Where specimen was at any given time
DEA Schedule

- Controlled Substances Act (1970)
  - I - High abuse potential, no medical use
    - Heroin, PCP, LSD, GHB, MDMA, etc.
  - II - High abuse potential, but has medical use
    - Most opioids, barbiturates, methylphenidate, etc.

DEA Schedule

- III - Ketamine, buprenorphine,
- IV - BZD, long acting barbiturates, modafinil
- V - Codeine cough suppressants, pregabalin, diphenoxylate

Medical Review Officer

- Licensed certified physician
- Responsible for receiving and reviewing lab results generated by a drug testing program
- Liaison between employer, lab and donor
- Act as an independent and impartial "gatekeeper" and advocate for the accuracy and integrity of the drug testing process
Drug Abuse Testing
- Strict, invariable procedures, federally mandated (DOT)
- Certified lab, separate from all other testing
- MRO interpretation required

Drug Abuse Testing
- 'SAMHSA 5' - cocaine, opioids, amphetamine, THC, PCP
- ETOH breath testing
- Screening, then confirmatory if + by cutoffs

Postmortem Toxicology

Postmortem Toxicology of Abused Drugs
Postmortem Toxicology

- Autolysis – 1st stage decomposition
- Cellular integrity fails
- Enzymes released
- Putrefaction – 2nd stage

Postmortem Toxicology

- Digestion of tissue by bacteria
- Skin/organ color changes
- Epithelial blebs
- Bloating from gas accumulation

Postmortem Toxicology

- Mummification – warm, dry climates
- Putrefaction does not occur
- Adipocere – from anoxic decomposition
- Anthropophagia
- Insects/animals feed on remains
- Embalming – chemically preserving tissues
Necrokinetics

- Change in drug concentration over time after death
- "Postmortem changes" or "redistribution"
- Goal: Did the drugs measured play a role in the patient's death?

Factors Affecting Drug Concentration

- Passive concentration-gradient-driven diffusion fluid shifts
- pH
- Apparent volume of distribution
- Condition of the body
- High cardiac to blood drug ratios' = high potential for redistribution

<table>
<thead>
<tr>
<th>Routine</th>
<th>Infrequent</th>
<th>Uncommon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bile</td>
<td>Bone</td>
<td>Antemortem blood</td>
</tr>
<tr>
<td>Blood</td>
<td>CSF</td>
<td>Extravasated blood</td>
</tr>
<tr>
<td>Brain</td>
<td>Fat</td>
<td>Extravasated fluid</td>
</tr>
<tr>
<td>Liver</td>
<td>Hair</td>
<td>Casket fluid</td>
</tr>
<tr>
<td>Gastric contents</td>
<td>Kidneys</td>
<td>Insect larvae</td>
</tr>
<tr>
<td>Urine</td>
<td>Lungs</td>
<td>Pupae casings</td>
</tr>
<tr>
<td>Vitreous humor</td>
<td>Muscle</td>
<td>Soil</td>
</tr>
<tr>
<td></td>
<td>Nails</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Skin</td>
<td></td>
</tr>
</tbody>
</table>
Sample Matrix

- Blood in forensics, serum in clinical (ie ethoh values differ 1.15)
- High Vd > 3 L/kg have high probability of redistribution
- ETOH formation through fermentation (usually 3 to 10 days)

Vitreous

- EtOH to blood: 0.9 to 1.3
- Vitreous EtOH lags behind BAC over time
- Aid in quantitative analysis of 6 acetylmorphine, benze, and electrolytes

Entomotoxicology

<table>
<thead>
<tr>
<th>Xenobiotics Reported from Larvae and Pupae Casings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzytocognine</td>
</tr>
<tr>
<td>Cocaine</td>
</tr>
<tr>
<td>Heroin</td>
</tr>
<tr>
<td>Malathion</td>
</tr>
<tr>
<td>Mercury</td>
</tr>
<tr>
<td>Methamphetamine</td>
</tr>
</tbody>
</table>
Interpretation Confounders

- Postmortem redistribution
- Postmortem metabolism
- Continued absorption
- Xenobiotic stability
- Chemical interactions
- Expected clinical effects
- Comorbid, tolerance, genetics

Redistribution Doesn't Occur

- Alcohols
- Carbon Monoxide
- Carbamazepine
- Chloral Hydrate
- Diflunisal
- Ephedrine
- Hydrocodone
- Hydroxyzine
- Lorazepam
- Lamotrigine
- Mirtazapine
- Nitrazepam
- Phenergan
- Procyclidine
- Quinidine
- Theophylline
- Zopiclone

Legal Ethanol

- State determines own legal driving limit
- DWI, DUI, DWAI, etc.
- Zero tolerance
- "Illegal per se"
- What is "drunk"?
- ~ 150 mg/dL
The Barman’s Paradox

- Dram shop is a legal term in the United States referring to a tavern where alcoholic beverages are sold
- Motor Vehicle 80 mg/dL
- Can be held liable serving the intoxicated
- No better at determining “drunk”

Blood Ethanol Testing

- The law = whole blood
- The lab = serum / plasma
- [serum] = [plasma]
- ETOH does not enter RBCs well
- [serum]/[blood] ~ 1.15
- Hospital serum lab measurement higher than the law

What’s in a Drink?

- 12 oz beer (5%)
- 5 oz of wine (12%)
- 1.5 oz liquor (40%)
- All = 14 g ETOH
- Avg adult: 15-20 mg/dL/hr
- Tolerance: 30 mg/dL/hr
Lab Methods for ETOH

- Enzymatic
- ADH + ETOH = NADH (340 nm)
- False+ with elevated lactate
- GC
- Can detect other volatiles ("toxic")

The Numbers

- Grams of Ethanol in a beverage
- F x V (ml) x 0.8 g/ml
- Vd 0.6 L/kg v 0.5L/kg

Sample Calculation

70 kg man drinks 2 standard beers
12 oz (30 ml/oz) x2 = 720 ml —— 5% of 720 ml = 36 ml
36 ml X 0.8 g/ml = 28.8 g which is 28,800 mg
70 kg (0.6 L/kg) = 42 L (10 dL/L) = 420 dL
28,800 mg/420 dL = 68.5 mg/dL
Breathalyzer

- Henry Law: ratio between concentration of ethanol in the alveolar air and the blood is constant
- Mean breath to blood ratio is 1:23000
- 1:21000 used in forensics

Breathalyzer

- Use electrochemical sensors for ethanol oxidation or infrared spectral analysis
- \([\text{Breath ethanol}] \text{ mmol/L} \times 2100 = [\text{Blood ethanol}] \text{ mmol/L}\)
- Breath units underestimate BAC

Breathalyzer Interference

- Belching/vomiting (ethanol in stomach)
- Inadequate exhalation
- Obstructive pulmonary disease
Breathalyzer Interference

- Mouth ethanol retained in the bridges or periodontal spaces
- Multidose inhales (Primatene Mist™ 34% ethanol)
- Mouthwashes (Listerine™ 27% ethanol, Scope™ 19% ethanol)