Analytical and Forensic Toxicology

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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
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- Poor sensitivity
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- Cross reactivity
- Calibration
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Performance Challenges

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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
Immunoassays

- 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
<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) 
\((-\)-\textit{trans-}Δ\textsubscript{9}-tetrahydrocannabinol

- Immunoassay: Inactive metabolite 11 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
- Bupropion
- 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, herbals
  - 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
  - Cylcobenzaprine
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
    - Falls 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

Anion gap (unmeasured anions)

- 95% cations measured
- 85% anions measured
- normal gap 7 +/- 4

MIND THE GAP
Low Anion Gap

- Hypercalcemia
- Hypermagnessemia
- Hyperkalemia
- Lithium
- Multiple myeloma
- Hyperalbuminemia
- Bromism
- Iodism
- Nitrate excess
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

- 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 patients 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 etoh 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, benzo, and electrolytes
Entomotoxicology

Xenobiotics Reported from Larvae and Pupae Casings

<table>
<thead>
<tr>
<th>Benzoylecognine</th>
<th>Morphine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocaine</td>
<td>Nortriptyline</td>
</tr>
<tr>
<td>Heroin</td>
<td>Oxazepam</td>
</tr>
<tr>
<td>Malathion</td>
<td>Phenobarbital</td>
</tr>
<tr>
<td>Mercury</td>
<td>Triazolam</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td></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
- Chlordiazepoxide
- Diflunisal
- Ephedrine
- Hydrocodone
- Hydroxyzine
- Lorazepam
- Lamotrigine
- Mirtazepine
- Nitrazepam
- Phenelzine
- 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
- \([\text{serum}] = [\text{plasma}]\)
- ETOH does not enter RBCs well
- \([\text{serum}]/[\text{blood}] \sim 1.15\)
- 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
- $\text{ADH} + \text{ETOH} = \text{NADH} \ (340 \text{ nm})$
- False+ with elevated lactate
- GC
- Can detect other volatiles (“toxic”)
The Numbers

- Grams of Ethanol in a beverage
- $F \times V \text{ (ml)} \times 0.8 \text{ g/ml}$
- $V_d \ 0.6 \text{ L/kg} \ 0.5 \text{L/kg}$
Sample Calculation

70 kg man drinks 2 standard beers

12 oz (30 ml/oz) x2 = 720 ml \[\rightarrow\] 5% of 720 ml = 36 ml

36 ml \times 0.8 \text{ g/ml} = 28.8 \text{ g which is 28,800 mg}

70 kg (0.6 \text{ L/kg}) = 42 \text{ L} (10 \text{ dL/L}) = 420 \text{ 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% etoh)
- Mouthwashes (Listerine™ 27% etoh, Scope™ 19% etoh)