Analytical and Forensic Toxicology

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Special Thanks!

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- Jeffrey Brent
- Adhi Sharma
- Chuck McCay
- Kent Olson

It was the break they had been waiting for; prints left at the crime scene
Content

• Core Content of Medical Toxicology
• Part 5: Analytical and Forensic Toxicology
• LLSA Articles
• Is this urine really negative? J of Sub Abus Treat 207;33:33-42.
Laboratories

- Clinical Laboratory Improvement Amendments of 1988 (CLIA)
- Medical Lab Testing governed by federal regulations since 1992
- Regulations apply to all lab testing of human specimens for medical purposes
- All tests require the possession of an appropriate certificate
Testing

- 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
Low volume, often old methodologies
• Limits to every technique
• Implies confirmation or exclusion
• More toxic xenobiotics than named diseases
Magic Answer Box?

- Low volume, often old methodologies
- Limits to every technique
- Implies confirmation or exclusion
- More toxic xenobiotics than named diseases
Basics of analysis

- Screening
- Confirmation
- Separation
- Detection
- Quantification
Assay Methods

- Spot & spectrochemical
- Immunoassays
  - Non-competitive
  - Competitive
- Enzyme-multiplied immunoassay (EMIT)
- Magnetic microparticle chemiluminescent competitive immunoassay
- Microparticle capture immunoassay
Assay Methods

- Chromatography
- Thin layer liquid (TLC)
- High performance (pressure) liquid (HPLC)
- Gas chromatography (GC)
- Mass spectroscopy (MS)
- Inductively coupled plasma mass spectroscopy
“You’re fired, Jack. The lab results just came back, and you tested positive for Coke.”
Spot tests

- Simple
- Rapid Reaction
- Color change
- Ferric chloride for salicylate
- Unreliable (FP & FN)
- Visual interpretation
Spot Testing

- Salicylates
- Ferric chloride
- Mercuric chloride
- Meixner Test
Spectrochemical

• Sophisticated spot tests

• Chemical reaction to form light-absorbing substance

• Carefully controlled

• Spectrophotometer vs eyeball
Co-oximetry

- Spectrophotometry used to measure various forms of hemoglobin
- Measurement of light absorbance at multiple wavelengths allows several hemoglobin species to be quantified
- Need more wavelengths than types of hemoglobin
Spectrochemical

- Older tests waited until complete conversion
- Modern tests measure rate of conversion
  - Initial phase, rate is constant and proportional to the initial concentration of the analyte
- Nonreacting substances that absorb light
  - Don’t affect results
Spectrochemical

- Produce light absorbing substances
  - Ex: High concentrations of lactate
- Inhibit the assay reaction or that consume reagents
  - Ex: ascorbic acid in oxidation reactions

Oh, a drug test. That’s a relief. I thought you were going to test my ethics.
Spectrochemical

- Improve Selectivity
- Enzymes that can catalyze highly selective reactions
- Assays for ethanol use alcohol dehydrogenase
- Rate of change of NAD$^+$ to NADH
- Rate of increase in light absorption is proportional to ethanol concentration
Immunoassays

- Created due to need to measure a very low concentration of an analyte
- Quick, inexpensive
- Combination of
  - High affinity
  - High selectivity
- 2 common types
Immunoassays

- Non-competitive
  - Analyte is sandwiched b/w 2 antibodies
  - Difficult with small drugs
- Competitive
  - Analyte from specimen competes for number of Ab binding sites with a labeled version of the analyte
Non-competitive

“Sandwich” assay
Competitive

“Inverse” assay
Competitive

From Goldfranks 9th edition
Immunoassays

- Nonisotopic immunoassays are common
- Limited to those in high demand
- Lots of effort, high development cost
- Low production cost
- Homogenous immunoassays
- Measure differences in bound and free labels
Immunoassays

- Old - radio immunoassays
- New - spectrophotometric
  - Enzyme multiplied immunoassay technique (EMIT)
  - Fluorescence polarization immunoassay (FPIA)
  - Kinetic inhibition of microparticles in solution (KIMS)
  - Cloned enzyme donor immunoassay (CEDIA)
EMIT

Goldfranks 9th edition
Chromatography
Separation and Detection

- Encompasses several related techniques where analyte specificity is achieved by physical separation

- Partition analytes between a stationary and mobile phase
  - Stationary: very fine particles arranged in a thin layer
  - Mobile (moving): phase flows through spaces between particles

- After separation—need a detection phase
  - However, can have provisional identification based on their characteristic velocities, distance traveled, or time to traverse the chromatography column

- Not specific
\[ R_f = \frac{\text{migration distance of substance}}{\text{migration distance of solvent front}} \]

- Retention time
- Time required to traverse the column
- Retardation (planar) or retention (column)
- Separation based on polarity, affinity, solubility, etc.
- Standard for each analyte
Thin Layer Chromatography

- Extracts dissolved in a solvent
- Placed on thin layer of silica gel
- Plates placed vertically in closed tank
- Solvent drawn upwards thru the gel
  - Xenobiotics are drawn up the gel
  - Hydrophobic rapidly
  - Hydrophilic slowly
TLC

Goldfranks 9th edition
Thin Layer Chromatography

- Fast, easy, inexpensive
- Polar silica medium
- Low sensitivity (~ 1000 ug/L)
- Low specificity
- Used in drug screens
- Requires large amount of material
- Done on urine, gastric aspirate
TLC Drawbacks

- Multiple steps
- Slow, labor intensive
- Interpretation of spots
- Difficult to quantitate
- Used to demonstrate the presence of a drug
High-Performance Liquid Chromatography

- Stationary phase packed in a column
- Mobile phase pumped through under pressure
- Allows better separation in less time
- Identification by retention time in a column
- Also use ultraviolet spectroscopy
- Measuring light absorbance allows the amount of the xenobiotic to be determined
Reverse phase chromatography

- Non-polar stationary phase and hydrophilic mobile phase
- Polar out first, non-polar retained
- Hydrophobic stationary
- Hydrophilic mobile phase
- Most common HPLC technique
- TLC can be done this way too
High-Performance Liquid Chromatography

- Expensive, complex, fast
- Separation under pressure
- Quantitation, detection > 10 ug/L (10 fold difference)
- Low specificity for same class
- One at a time; narrow range of polarity
- Measure serum concentration for which no immunoassay is available
- Can’t analyze multiple specimens
HPLC
Gas Chromatography

- Similar to HPLC except the moving phase is a gas
  - Nitrogen, helium
  - Low flow resistance of gas
  - High flow rates
  - Less time

- Temperature gradient allows multiple xenobiotics to be analyzed at once
  - Partitioning depends on natural volatility

- Temp < 572°F (300°C)
Gas Chromatography

- Expensive
- Screening or confirmatory
- High specificity and sensitivity
- Quantitation and broad screening
- Temperature gradient
- Analyte volatilized in injector, column kept hot
- Heat labile cannot be assessed
Gas Chromatography

- Substances have characteristic retention times
- Column outflow detectors
  - Flame-ionization - organic compounds
  - Most common detector
  - Nitrogen-phosphorous - N or P compounds (many drugs)
Mass Spectrometry

- Can serve as highly sensitive GC detector
- Analyte separated from the gas carrier and then filtered to a detector
- Extremely high specificity
- Unparalleled ID of organic chemicals
- Shoots electrons → fragment ions
  - Characteristic of the parent molecule
Mass Spectrometry
MS Limits

- Cannot separate enantiomers
- D-methamphetamine (DOA)
- L-methamphetamine (inhalers)
- Vick’s inhalers, metabolite of selegiline
- “L” = legal
- Can separate by a chiral analysis
• High sensitivity and specificity extended by the related hybrid technique of liquid chromatography/tandem mass spectrometry
GC/MS

- Expensive equipment
- Most common gold standard, in ~1 hour
- Most specific and sensitive
- Sensitivity 2 – 10 ug/L
# Relative Comparison

<table>
<thead>
<tr>
<th>Method</th>
<th>Speed</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spot Test</td>
<td>Fast</td>
<td>$</td>
</tr>
<tr>
<td>Spectrochemical</td>
<td>Medium</td>
<td>$</td>
</tr>
<tr>
<td>Immunoassay</td>
<td>Medium</td>
<td>$$</td>
</tr>
<tr>
<td>TLC</td>
<td>Slow</td>
<td>$$</td>
</tr>
<tr>
<td>HPLC</td>
<td>Medium</td>
<td>$$</td>
</tr>
<tr>
<td>GC</td>
<td>Medium</td>
<td>$$</td>
</tr>
<tr>
<td>GC/MS</td>
<td>Slow</td>
<td>$$$</td>
</tr>
<tr>
<td>LC/MS/MS</td>
<td>Medium</td>
<td>$$$$</td>
</tr>
</tbody>
</table>

*Reproduced from Goldfranks 9th edition*
Hair Analysis

- Hair grows ~1 cm/month
- Can utilize any methodology
- Contamination is greatest flaw
Hi, i know your a teenager, i know your friends smoke weed, i need some.

SURE! How much you need? I got some in my room if you pay me.

Dumbass, that was a test.

Grounded?

Get home. now.
NIDA-5

- Amphetamines
- Cannabinoids
- Cocaine
- Opiates
- Phencyclidine
Cocaine

- Highly specific
- Benzylecgonine
- Inactive metabolite
- No false positives
- Acute: 2-3 days
- Chronic: 1 week
Tetrahydrocannabinol

- Tetrahydrocannabinolic acid
- Inactive metabolite
- Occasional use: 3 days
- Chronic use: > 1 month
- 11-OH THC
The “second hand smoking” defense

- Studies of passive exposure
- Confined areas
- Mixed results
- Cutoffs: 20 ng/ml
- Levels: ~6 ng/ml
- NIDA 50 ng/ml
THC

- Dronabinol
- Efavirenz
- NSAIDs
- Promethazine
- Riboflavin
- Ethacrynic acid
Opioids

- Morphine, codeine, heroin
- Cross reactivity depends on assay
- Synthetics show little or no cross-reactivity
- Specific directed assays available
“Poppy Seed” Defense

6-MAM
Challenges

Dextromethorphan

• Dextrorphan – major metabolite
• Levorphanol – “L” enantiomer, also opioid
• Can’t differentiate optical enantiomers by MS
Methadone

False Positives

- Quetiapine
- Doxylamine
- Olanzapine
- Diphenhydramine & Verapamil metabolites

*Tests for other synthetic and semisynthetics are available
PCP

- Phencyclidine
- False Positives
  - DXM, Ketamine
  - Diphenhydramine
  - Venlafaxine, bupropion
- Metabolites of PCP
- False Negatives?
Benzodiazepines

- Metabolized to oxazepam
- Chlordiazepoxide
- Diazepam
- Temazepam
- Glucuronides (lorazepam)
Amphetamines

- Amphetamine assay plagued with false positives
- Fails to detect “designer” amphetamines
- Bupropioin (cathinone)
- Pseudoephedrine
Nasal Inhalers

- Can contain $l$-methamphetamine
- less potent isomer of $d$-methamphetamine
- Both turn immunoassays positive
- Difficult to distinguish with mass spec
- Optical enantiomers
TCA Challenges

- Cross react with ringed xenobiotics
  - Carbamazepine
  - Phenothiazines
  - Diphenhydramine

- Major challenge is timing
## 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; 1000 ng/ml THCA</td>
<td>15 ng/ml THCA</td>
</tr>
<tr>
<td>PCP</td>
<td>25 ng/ml</td>
<td>25 ng/ml</td>
</tr>
</tbody>
</table>
Adulteration

- 2004: First mandatory guidelines for federal workplace testing (SAMHSA)
- Tampering
  - In vivo adulteration
  - In vitro adulteration
- Urine substitution
- Little evidence to support any product working consistently
Specimen Validity

- Appearance
- Temperature
  - 90-100°F
- pH testing
  - pH 3-11
- Specific gravity
  - Cr < 20 mg/dl
  - > 1.003
- Creatinine
  - > 20 ppm
In Vivo Adulteration

“Ingest prior to urination”

- Primary mechanisms: dilution and excretion
- Water and diuretics
- Fool visual inspection
- Interfere with creatinine level checks
- Water, Naturally Klean herbal tea, golden seal, HCTZ
- B-vitamins, riboflavin, creatinine
- Riboflavin may cause fluorescent urine
In Vitro Adulteration

“Add after urination”

• Interfere with an immunoassay or convert a target drug to a different compound

• Sold under many names but contain:
  • Glutaraldehyde
  • Sodium or potassium nitrate
  • Pyridinium chlorochromate
  • Peroxide/peroxidase
  • Household products, too
In Vitro Adulteration

“Add after urination”

- Glutaraldehyde: interferes with immunoassay, denatures
- Nitrite: decreased ion concentration
- Pyridinium: decrease in pH levels
- Peroxidase: oxidizing drugs and metabolites
- Bleach: decreases detection
- Vinegar: interferes with detection; lowers pH
Others

- Acids and bases (i.e. lemon juice, NaOH)
- Oxidizing agents (i.e. bleach, peroxide)
- Denaturants (i.e. glutaraldehyde)
- Eyedrops (i.e. benzalkonium chloride)
  - Reduces binding of immunoassay
- Soap (created FP and FN)
Urine Substitution

Ontario Smith
Cholinesterases

- Butyrylcholinesterase (plasma cholinesterase)
  - Metabolizes cocaine, succinylcholine
  - Falls first, recovers first
- Red Cell Acetylcholinesterase
  - Reflects activity at the NMJ
  - Low concentrations in people
- Serve as markers for poisonings
Cholinesterases

Butrylcholinesterase
- Malnutrition
- Hereditary deficiency
- Iron deficiency anemia
- Hepatic disease
- Chronic Illness
- Succ, codeine, morpine

Red Cell
- Pernicious anemia
- Antidepressants
- Antimalarials
- Hemoglobinopathies
- Inc with oral contraceptives

Malnutrition
Hereditary deficiency
Iron deficiency anemia
Hepatic disease
Chronic Illness
Succ, codeine, morpine
Pernicious anemia
Antidepressants
Antimalarials
Hemoglobinopathies
Inc with oral contraceptives
Anion Gap Reliability

AG = Na⁺ - (Cl⁻ + HCO₃⁻)

- **MUDPILES**
  - Cyanide, CO, Acetaminophen, Toluene, Theophylline, Hydrogen Sulfide
  - Increase unmeasured anions
  - Dehydration, sodium salts, antibiotics
- Decreased unmeasured cations
  - Mag, Ca, and K
Anion Gap Reliability

Low Anion Gap

- Hypercalcemia
- Hypermagnesemia
- Hyperkalemia
- Lithium
- Multiple myeloma
- Hypoalbuminemia
- Bromism
- Iodism
- Nitrate excess
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.
  - No refills
- III - Ketamine, buprenorphine, GHB
- IV - BZD, long acting barbiturates, modafinil
- V - Codeine cough suppressants, pregabalin, diphenoxylate
Medical Review Officer

- Licensed physician
- “Expert in drug and alcohol testing and the application of federal regulations to the process.”
- Consultant
  - Business, industry, labor, government or academia
- Relating to prevention, detection and control of drug abuse in the workplace
Drug Abuse Testing

- Strict, invariable procedures, federally mandated (DOT)
- Certified lab, separate from all other testing
- MRO interpretation required
- COAT-PE
- ‘SAMHSA 5’ - cocaine, opioids, amphetamine, THC, PCP
- ETOH breath testing
- Screening, then confirmatory if + by cutoffs
Post Mortem Toxicology
Post Mortem Changes

“Necrokinetics”

- Postmortem interval
- Defined by the degree of decomposition
- Decomposition
  - Autolysis: enzymes are released and chemicals move down gradients
  - Putrefaction: digestion by bacteria
  - Anthropophagia: feeding on remains
Specimens

- Blood
  - Reported as “blood concentrations”
  - From femoral or subclavian (low glucose)
  - Right heart blood (elevated glucose)

- Vitreous
  - Avascular, acellular so well protected
  - Aqueous content > blood

- Urine
  - Bladder serves as a “reservoir”
Interpretation Confounders

- Postmortem redistribution
- Postmortem metabolism
- Continuous absorption
- Xenobiotic stability
- Chemical interactions
- Expected clinical effects
- Comorbid, tolerance, genetics
Other sources
Redistribution Doesn’t Occur


<table>
<thead>
<tr>
<th>Table 1. Toxins/drugs in which postmortem redistribution probably does not occur.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol³</td>
</tr>
<tr>
<td>Carbon monoxide</td>
</tr>
<tr>
<td>Carbamazepine</td>
</tr>
<tr>
<td>Chlor Diazepoxide</td>
</tr>
<tr>
<td>Diflunisal</td>
</tr>
<tr>
<td>Ephedrine</td>
</tr>
<tr>
<td>Hydrocodone</td>
</tr>
<tr>
<td>Hydroxyzine</td>
</tr>
<tr>
<td>Lorazepam</td>
</tr>
<tr>
<td>Lamotrigine</td>
</tr>
</tbody>
</table>
Man eats underwear to beat breathalyzer

By D’ARCY RICKARD of The Advocate

STETTLER — An 18-year-old Stettler man tried to eat his underwear in the hope that the cotton fabric would absorb alcohol before he took a breathalyzer test, provincial court heard this week.

was removed by the teacher when testimony enlivened the proceedings. The Grade 11 and 12 students had difficulty maintaining composure.

“People were leaving the courtroom with tears in their eyes, trying not to laugh,” said RCMP Const. Peter McFarlane.
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

- Legally drunk 50-80 mg/dL or 0.05-0.08 g/dL [%]
- Serving to intoxicated is prohibited
- 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
  - $[\text{serum}]/[\text{blood}] \sim 1.15$
- The lab measures higher than the law
- $[\text{Breath ethanol}] \text{ mmol/L} \times 2100 = [\text{Blood ethanol}] \text{ mmol/L}$
The Clues

1. Wide turns
2. Straddling / driving on lane marker
3. Nearly striking object or another vehicle
4. Weaving / swerving
5. Going too slow (> 10 MPH below speed limit)
6. Stopping inappropriately / without cause
7. Following too closely
8. Erratic braking
9. Driving into opposing / crossing traffic
10. Slow response to traffic signals

*NHTSA Top 10 in descending order of probability of intoxication*
<table>
<thead>
<tr>
<th>Serum Ethanol Concentration</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 mg/dL (4.35 mmol/L)</td>
<td>Impairs driving-related skills</td>
</tr>
<tr>
<td></td>
<td>Gross motor control and orientation may be significantly affected</td>
</tr>
<tr>
<td>50 mg/dL (10.87 mmol/L)</td>
<td>Clinical ethanol intoxication is usually apparent</td>
</tr>
</tbody>
</table>

Goldfranks 9th edition
What’s in a Drink?

Approximately

- 10 oz beer (5%)
- 4 oz of wine (12%)
- 1 oz liquor (50%)
- All ~ 15 g ETOH
- 100-125 mg/kg/hr
- Avg adult: 7-10 g/hr or 15-20 mg/dL/hr
- Tolerance: 30 mg/dL/hr
Laboratory Methods

- Enzymatic
- \( ADH + ETOH = NADH \) (340 nm)
- FP with elevated lactate (lactate converted to pyruvate increases NADH formation)
- GC
- Can detect other volatiles ("toxic")
The Numbers

- Specific gravity 0.8 g/ml
- Vd 0.6 L/kg
- 1-1.5 shot(s) = 30 ml
- mmol/L = (mg/dL)/4.6
- % = grams/100 ml
Sample Calculation

50 kg man drinks 1 shot of 80 proof alcohol

1.5 oz (30 ml/oz) = 45 ml → 40% of 45 ml = 18 ml

18 ml × 0.8 g/ml = 16 g which is 16,000 mg

50 kg (0.6 L/kg) = 30 L (10 dL /L) = 300 dL

16,000 mg/300 dL = 80 mg/dL
“Breathanol”

- [expired air] surragote for [blood] (sort of)
- Henry’s law
- [ethanol] remains constant throughout respiratory tract
- Mean breath:blood ratio 1:2100 used in forensics
- Many confounders
- [Breath ethanol] mmol/L \times 2100 = [Blood ethanol] mmol/L
- Breath units underestimate BAC
References

1. Goldfranks 9th Edition


Photo References