Clinical Drug Testing
and Diagnostics

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Basics of analysis

- Screening
- Confirmation
- Separation
- Detection
- Quantification

Assay Methods

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

- Chromatography
  - Thin layer liquid (TLC)
  - High performance (pressure) liquid (HPLC)
  - Gas chromatography (GC)
- Mass spectroscopy (MS)
  - Inductively coupled plasma mass spectroscopy

**Spot tests**

- Simple
- Rapid Reaction
- Color change
- 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

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 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 between 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
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

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
- GOD-labeled drug
- Unlabeled drug
- Anti-drug antibody
- NAD⁺
- NADH

Goldfranks
Chromatography

- Think separation and detection
- 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 thru 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

Retention Time (Rf)

\[ Rf = \frac{\text{migration distance of substance}}{\text{migration distance of solvent front}} \]

- 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
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 ng/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**

- 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)

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**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
**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

**LC/MS/MS**

- 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>

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### Cholinesterases

- Normal ranges of RBC and serum cholinesterase vary widely between individuals
- Rarely available in time to guide the emergency treatment decisions
- Cholinesterase levels often compared with the general population range
- Butyrylcholinesterase (plasma cholinesterase, PCE)
  - Plasma, liver, pancreas, and the white matter of the brain
  - Falls first, recovers first
- RBC Acetylcholinesterase
  - Responsible for the hydrolysis of ACh at nerve endings
  - Reflects activity at the NMJ
Butyrylcholinesterase (PCE)

- Low
  - Illness
  - Benzalkonium salts
  - Carbon disulfide
  - Ciguatoxin
  - Cocaine
  - Morphine
  - Oral contraceptives
  - Organic mercury
  - Solanine
  - Succinylcholine

- High
  - Nephrotic syndrome

PCE Low Level Fun Facts

- Gray-top blood tubes or those containing fluoride
- Genetic variant (6-7% in surgical populations)
  - Lacks ability to hydrolyze ester bonds
  - Usually associated with normal RBC cholinesterase
  - Dibucaine test

Red Cell Cholinesterases

- Low
  - Antimalarial drugs
  - Oral contraceptives
  - Some anemias
**Biological monitoring**

<table>
<thead>
<tr>
<th>Substance</th>
<th>Biological marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>Urine phenol</td>
</tr>
<tr>
<td>Toluene</td>
<td>Urine hippuric acid</td>
</tr>
<tr>
<td>Styrene</td>
<td>Urine mandelic and phenylglyoxylic acid</td>
</tr>
<tr>
<td>Radon</td>
<td>EPA's action level 2 pCi/L</td>
</tr>
<tr>
<td>Xylene</td>
<td>Urinary Methylhippuric acid</td>
</tr>
<tr>
<td>Carbon disulphide</td>
<td>Urine 2-thiazolidine-4-carboxylic acid (TTCA)</td>
</tr>
<tr>
<td>Polyaromatic HC</td>
<td>Urine 1-hydroxypyrene</td>
</tr>
<tr>
<td>Isocyanates</td>
<td>Isocyanate-derived diamines</td>
</tr>
</tbody>
</table>

**High Yield Concepts**

- How do you measure alcohols?
  - Gas chromatography

- What types of xenobiotics require testing beyond a point-of-care testing format?
  - Drugs with small concentrations

- Gold standard for confirming a drug of abuse?
  - GC/MS, LC/MS/MS

- Immunoassays are presumptive but highly sensitive and specific

**High Yield Concepts**

- Presence of a drug in lab testing does not necessarily establish the presence of clinical toxicity
- Immunoassays do not require chemical modification of the drug/sample
- Time of ingestion for drugs of abuse is not typically necessary for proper interpretation of the test result
- Plasma cholinesterases fall and rise faster
- Breath tests are considered patient monitoring and not subject to laboratory regulation