What Is Forensic Toxicology

Vina Spiehler, PhD, DABFT
Forensic Toxicology:

- Forensic toxicology is the science of poisons which deals with their isolation, identification and quantification from tissue and in the interpretation of findings.
- These findings are used in establishing cause of death, clarifying circumstances surrounding death or identifying the level of impairment of drug intoxicated drivers.
- Robert Cravey 1981
What Questions Can Forensic Toxicology Answer:

• Were drugs or alcohol involved
• How much did they take?
• When did they take it?
• Did it kill them?
• How certain are you?

• Issues: Sensitivity, Specificity, Confirmation and Certainty
Forensic Pharmacology

- **Pharmacodynamics**
  - What the drug does to the body
    - Intoxication

- **Pharmacokinetics**
  - What the body does to the drug
    - Metabolism
Case 1

- 23 y o M found in drivers seat. Car left roadway and collided with tree.

- Cause of death: traumatic head injury

- Toxicology results:
  - Femoral Blood: .20 g% ethanol
  - Heart blood: .19 g% ethanol
  - Vitreous fluid: .25 g% ethanol
## Stages of Ethanol Intoxication

<table>
<thead>
<tr>
<th>Blood Alcohol Level</th>
<th>Stage</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.09-0.25</td>
<td>Excitement</td>
<td>Emotional instability; loss of critical judgment; Impairment of perception, memory and comprehension; Decreased sensory response; increased reaction time; Reduced visual acuity and peripheral vision and slow glare recovery; Sensory-motor incoordination; impaired balance; slurred speech; Vomiting; drowsiness</td>
</tr>
<tr>
<td>0.18-0.30</td>
<td>Confusion</td>
<td>Disorientation, mental confusion; vertigo; dysphoria; Exaggerated emotional states (fear, rage, grief, etc.); Disturbances of vision (diplopia, etc.) and of perception of color, form, motion, dimensions; Increased pain threshold; Increased muscular incoordination; staggering gait; ataxia; Memory loss; Apathy; progressive lethargy</td>
</tr>
</tbody>
</table>
Case 2

- 20 yo F nude body found partially in river in red blanket.
- Cause of death undetermined.
- Toxicology results:
  - Immunoassay screening positive for opiates both blood and urine
  - Postmortem blood contained 0.45 mg/L free morphine and 0.70 mg/L total morphine (morphine and metabolites.)
- Hair Testing:
  - Immunosassay screen positive for opiates along entire length of hair in 5 cm segments
  - Hair next to scalp contained both morphine and 6-monoacetylmorphine
Confirmation

• First: Screening
  o Fast
  o Cheap
  o Very Sensitive

• Second: Confirmation
  o Accurate
  o Precise
  o Very Specific

• Result: Certainty
Confirmation

• In 1978 the Toxicology Section of the American Academy of Forensic Sciences resolved that “to be presented as evidence in court an immunoassay drug test positive result must be confirmed by a second method based on a different chemical or physical property of the analyte.”
Rosalyn Yalow Ph.D.
Radioimmunoassay

- Rosalyn Sussman Yalow
- 1921-2011 New York, NY
- 1950s Yalow and Berson developed RIA at the Bronx VA Hospital
- Published 1960 J Clinical Investigation
- 1976 Lasker Award Basic Medical Research
- 1977 Nobel Prize in Medicine and Physiology
- Consultant to Beckman Instruments
Radioimmunoassay

Radioimmunoassay for insulin, which would combine virtually absolute specificity with a high degree of sensitivity, sufficiency, and freedom from the usual non-specific factors, is now available.

The method involves the use of a labeled insulin antibody to measure insulin in plasma. The labeled antibody is added to a sample of plasma, and the unbound portion is separated from the bound portion by paper chromatography. The bound labeled antibody is then measured in a gamma counter.

The method has been found to be sensitive and specific, with a detection limit of 0.1 units per liter. It is also free from the usual non-specific factors that can affect the results of other methods.

The method has several advantages over other methods for measuring insulin. It is sensitive, specific, and free from the usual non-specific factors. It can be used to measure insulin in very small samples of plasma, and it is easy to use.

The method has been found to be reliable and reproducible, and it has been used to measure insulin in a variety of clinical situations. It is now widely used in clinical laboratories, and it is considered to be the gold standard for measuring insulin in plasma.

For years, investigators have sought an assay which would combine virtually absolute specificity with a high degree of sensitivity, sufficiency, and freedom from the usual non-specific factors. Now, such an assay is available. The method is simple, sensitive, specific, and free from the usual non-specific factors. It is now widely used in clinical laboratories, and it is considered to be the gold standard for measuring insulin in plasma.
Radioimmunoassay
Radioimmunoassay

labeled antigen $\text{Ag}^*$ (F) + specific antibody $\text{Ab}$ + unlabeled antigen $\text{Ag}^+$

labeled antigen-antibody complex $\text{Ag}^*$-$\text{Ab}$ (B) → unlabeled antigen-antibody complex $\text{Ag}$-$\text{Ab}$
Radioimmunoassay

Unlabeled antigen (ng)

cpm Bound/cpm Free

ratio in “unknown”

antigen in “unknown”

1/28/2016 ● TIAFT 50th Anniversary
Arnold O. Beckman

• 1900 IL- 2004 CA
• 1922 BA, MA U Illinois
• 1928 PhD Caltech Inst.
• 1934 Invented pH meter
• 1941 Invented DU UV-Vis spectrophotometer
• 1942 Invented IR-I
• 1943 B-P Oxygen Analyzer
• 1955 started Shockley Semiconductor Lab: heliopot, transistor
• 1977 Beckman Research Institutes
Beckman Instruments in the OCSD FSS Lab

- Beckman pH Meter
- Beckman DU Spectrophotometer
- Beckman Model 2A Gas Chromatograph
- Cadman-Johns Blood Alcohol Procedure
Beckman RIA and EIA in the OCSD FSS Lab

- Beckman BioGamma
- Spinco Centrifuge
- Spinco Microfuge
- Beckman Digoxin RIA
- Roche Morphine Assay
- Spiehler, V. Drugs of Abuse Radioimmunoassay Directory
- Beckman 24/25 Spectrophotometer
Radioimmunoassay

* Spiehler, VR. Drugs of Abuse Radioimmunoassay Directory. Clinical Toxicology 8: 257-265, 1975

- Spiehler, Reed, Cravey, Wilcox, Shaw and Holland. Comparison of results for quantitative determination of morphine by radioimmunoassay (RIA) enzyme immunoassay (EMIT) and spectrofluorometry. J Forensic Sci. 1975
- Spiehler, Sun, Miyada, Sarandis, Walwick, Klein, Jordan and Jessen. Radioimmunoassay, enzyme immunoassay, spectrophotometry and gas liquid chromatography compared for determination of phenobarbital and diphenylhydantoin. Clinical Chemistry 1976
- Selesky, Spiehler, Cravey and Elliott. Digoxin concentrations in fatal cases. J. Forensic Sci. 1977
# Radioimmunoassay

## Advantages
- More sensitive than Thin Layer Chromatography
- No sample preparation required
- Uses small sample volumes
- Low cost
- Can be automated for high volume screening
- eg Digoxin, Digitoxin

## Disadvantages
- Antibody cross-reactivity to related compounds
- Special precautions when handling radioactive substances
- False positive results due to interferences or cross-reactivity
- eg DLIRS Digoxin Like Immunoreactive Substance
Solid Phase Radioimmunoassay

- 1968 Pharmacia patent for solid phase immunoassays
- Simplifies separation of bound and free radio-label
- Washing step eliminates interference from post-mortem hemolysis and decomposition
1970 S Spector raises Abs to morphine (and morphine metabolites) by coupling it to a protein.

Broadly reactive Opiate tests v Specific Morphine reactivity depends on site of protein coupling.

I-125 label on coupled protein or tyrosine.

DPC Coat A Count antibody coated tube RIAs.

Technical Director Vina Spiehler.
ELISA: Solid Phase EIA
ELISA
Solid phase EIA

- Antibody or antigen can be bound to well
- Label can be an enzyme such as HRP
- Washing step removes color and interferences
- Substrate added and color read after incubation.
Cozart, Abingdon, UK
LFIA: Immunochromatography

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LFIC: Cozart Rapiscan

• Chris Hand, Dene Baldwin and Philip Hand
• 1996 SMART award for a non-invasive point of contact drug test
• 1998 Saliva swab lateral flow test strip with dye indicator
• Electronic readout
• 1999 Cozart RapiScan
• QuantiSal collector
LFIC: DDS

- 2006 Cozart DDS
- Non-invasive Roadside forensic drug test
- Oral fluid collector
- Faster times
- 2011 Alere Mobile DDS2
- 3 step, 1 min sampling time
- 5 drug, 5 min incubation
- THC cutoff 25 ng/ml
- CHP trials started in 2014
Queen’s Prize for DDS
Issues in Immunoassays in Forensic Toxicology

- Crossreactivity
- Confirmation
- Cutoffs, Sensitivity and Specificity
- Predictive Value and Certainty (Bayes Law)
Sensitivity and False Positives

- **Sensitivity is the true positive rate:**
  
<table>
<thead>
<tr>
<th>Test Positive</th>
<th>Test Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Drug Present</strong></td>
<td>48</td>
</tr>
<tr>
<td><strong>Drug absent</strong></td>
<td>1</td>
</tr>
</tbody>
</table>

  Sensitivity = \( \frac{TP}{TP + FN} = \frac{48}{49} = 98\% \)

- **Specificity is the true negative rate:**

  Sensitivity = \( \frac{TN}{TN + FP} = \frac{24}{25} = 96\% \)

Sensitivity and specificity depend on the cutoff concentration chosen as the decision threshold.
Receiver Operating Characteristic Plots

- Plotting sensitivity vs specificity for each decision threshold:
- Compare different diagnostic tests
- Choose optimal decision thresholds
Fig. 1. True positives, false negatives, false positives and true negatives vs. cutoffs for the Cozart® Amphetamine Microplate EIA for cutoffs at 10, 20, 30, 45, 50, 75 and 100 ng/ml amphetamine equivalents.

Fig. 2. Receiver operating curve: sensitivity vs. one minus the specificity for the Cozart® Amphetamine Microplate EIA for Oral Fluid. Data points are, from right to left for cutoffs at 10, 20, 30, 40, 45, 50, 60, 75, 100 ng/ml amphetamine equivalents. The data for
Bayes Law: Predictive Value

• \( P(H:E) = \frac{P(E:H)}{P(E:H) + P(E:\text{not}H)} \)

• **Predictive value of a positive test result** = 
  \[ \frac{\text{prevalence x sensitivity}}{\text{prevalence x sensitivity} + (1-\text{prevalence})(1-\text{specificity})} \]

• **Predictive value of a series of collaborating or confirming tests:** After each test, the predictive value of the preceding test becomes the prevalence for the next test in the series.
Bayes Law: Predictive Value

- Predictive value of a positive test result = 
  \[
  \frac{(\text{prevalence} \times \text{sensitivity})}{(\text{prevalence} \times \text{sensitivity}) + (1-\text{prevalence})(1-\text{specificity})}
  \]

- With a prevalence of 10%, the positive predictive value for a test with a sensitivity of 98% and a specificity of 96% is 73%. Negative predictive value is 99.7%
Bayes Law: Predictive Value

• Predictive value of a positive test result =
  \[
  \frac{(\text{prevalence} \times \text{sensitivity})}{(\text{prevalence} \times \text{sensitivity}) + (1-\text{prevalence})(1-\text{specificity})}
  \]

Test Sensitivity of 98% and a specificity of 96%

<table>
<thead>
<tr>
<th>Prevalence</th>
<th>10%</th>
<th>1%</th>
<th>0.1%</th>
</tr>
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<tbody>
<tr>
<td>Positive p.v.</td>
<td>73%</td>
<td>25%</td>
<td>3%</td>
</tr>
<tr>
<td>Negative p.v.</td>
<td>99.7%</td>
<td>&gt;99.9</td>
<td>&gt;99.9</td>
</tr>
</tbody>
</table>
Immunoassay in Forensic Toxicology

Conclusions

• Immunoassay screening has become an essential part of forensic drug testing.

• IA screening for drugs has provided a rapid low cost approach to roadside and workplace drug testing.

• Immunoassay results must be interpreted with caution.
In 1978 the Toxicology Section of the American Academy of Forensic Sciences resolved that "to be presented as evidence in court an immunoassay drug test positive result must be confirmed by a second method based on a different chemical or physical property of the analyte."
Case 2

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Case 2

Conclusions

• **Cause of death: heroin overdose.** Probability 75%
  - **Dose:** greater than 150 mg heroin

• **Time of use and time of death: Rapid**
  - Ratio of free to total morphine 64.3%: $0.45/0.70 = 0.643$
  - A ratio of greater than 50% indicates a rapid death. Probability 85%

• **Hair Testing:**
  - Chronic user of heroin from presence of morphine and 6-monoacetylmorphine along length of hair
  - Identified as missing person from neighboring country