

# Initial Testing for Drugs of Abuse in Hair Specimens

Prepared for  
DTAB

David A. Engelhart, Ph.D.

Laboratory Director, Omega Laboratories, Inc.

July 15, 2013

# Presentation Overview

- Cutoff Levels and Target Drugs
- Immunoassays for Hair Testing
- Sample Preparation Techniques
- Adulterants/Treatments
- Existing Standards

# Cut-off levels (pg/mg)\*

- \* 1pg = 1,000ng
- Carboxy-THC\*\* vs. THC\*\*\*
- Need for sensitive screening methods

<b>Drug Panel</b>	<b>Proposed 2004</b>	<b>SOHT</b>	<b>EU</b>
Cocaine	500	500	500
Opiates	200	200	200
Amphetamines	500	200	200
PCP	300	na	na
Marijuana	1**	100***	50***

# Cut-off levels (pg/mg) (Continued)

- Current laboratories with FDA-cleared screening assays

<b>Drug Panel</b>	<b>Omega</b>	<b>Quest</b>	<b>Psychemedics</b>
Cocaine	500	300	500
Opiates	300	500	200
Amphetamines	500	300	500
PCP	300	300	300
Marijuana	1	1	1

# Target Analytes

<b>Drug Panel</b>	<b>Target Analyte</b>
Cocaine	Cocaine
Amphetamines	Methamphetamine
Marijuana	Carboxy-THC
Opiates	Morphine
Synthetic Opiates	Oxycodone
PCP	PCP

# Immunoassays

- Assays which utilize antibodies to identify and measure amounts of drugs/metabolites
- Competitive binding process - labeled and unlabeled drug compete for antibody binding sites
- Comparison against drug standards of known concentrations allows for quantitation
- Heterogeneous vs. homogeneous assays

# Immunoassays (continued)

- Heterogeneous Assays
  - ELISA – (Enzyme-Linked Immunosorbent Assay) currently most common
    - Advantages
      - Provide needed sensitivity (pg/mg range)
      - Specificity – (d,l-methamphetamine)
      - Matrix effects are minimal
    - Disadvantages
      - Not easily adapted to high speed analyzers
      - Requires wash step to separate bound and free antigen
        - Time & labor costs

# Immunoassays (continued)

- Homogeneous Assays
  - Advantages
    - Do not require separation step
    - Can be fully automated
      - High throughput
      - Low labor costs
  - Disadvantages
    - Sensitivity
    - Specificity
    - Matrix effects

# Immunoassays (Continued)

- All positive screening results must be confirmed
  - (GC/MS, GC/MS/MS or LC/MS/MS)
- Proficiency Testing Programs demonstrate the effectiveness of different immunoassays
  - Sensitivity to meet detection requirements
  - Specificity
  - Precision around the cut-off
  - Cross-reactivity

# Omega study submitted for FDA 510(k) clearances

- Cocaine – ELISA Intra-Assay Precision using Spiked Samples (non-normalized data)

Cocaine Spiked Sample (n=11)	negative	125 pg/mg (-75%)	250 pg/mg (-50%)	375 pg/mg (-25%)	625 pg/mg (+25%)	750 pg/mg (+50%)	875 pg/mg (+75%)	1000 pg/mg (+100%)
Mean Abs. (450 nm)	2.285	1.690	1.282	1.049	0.675	0.589	0.492	0.432
S.D.	0.06298	0.05488	0.04112	0.02951	0.01436	0.01644	0.01660	0.00999
%CV	2.8	3.2	3.2	2.8	2.1	2.8	3.4	2.3

- Cocaine – ELISA Inter-Assay Precision using Spiked Samples (normalized data)

Cocaine Spiked Sample (n=222)	negative	125 pg/mg (-75%)	250 pg/mg (-50%)	375 pg/mg (-25%)	625 pg/mg (+25%)	750 pg/mg (+50%)	875 pg/mg (+75%)	1000 pg/mg (+100%)
Mean Abs. (450 nm)	2.282	1.666	1.316	1.080	0.731	0.645	0.552	0.499
S.D.	0.04977	0.07760	0.07897	0.06734	0.05859	0.05562	0.05408	0.05062
%CV	2.2	4.7	6.0	6.2	8.0	8.6	9.8	10.1

# Omega study submitted for FDA 510(k) clearances

- Cocaine – ELISA Summary of Agreement Study Results

ELISA Test Result (n=345)	Negative by GC/MS (less than 50 pg/mg)	Less than half the cutoff concentration by GC/MS	Near Cutoff Negative (Between 50% below the cutoff and the cutoff concentration)	Near Cutoff Positive (Between the cutoff and 50% above the cutoff concentration)	High Positive (Greater than 50% above the cutoff concentration)
Positive	0	0	31	23	165
Negative	122	2	2	0	0

- Cocaine – ELISA Summary of Agreement Study Results

ELISA Test Result (n=345)	Less than half the cutoff concentration by GC/MS	Near Cutoff Negative (Between 50% below the cutoff and the cutoff concentration)	Near Cutoff Positive (Between the cutoff and 50% above the cutoff concentration)	High Positive (Greater than 50% above the cutoff concentration)
Positive	0%	9.0%	6.7%	47.8%
Negative	0.6%	0.6%	0%	0%

# Omega study submitted for FDA 510(k) clearances

- THCA – ELISA Intra-Assay Precision using Spiked Samples (non-normalized data)

THCA Spiked Sample (n=10)	negative	0.25 pg/mg (-75%)	0.5 pg/mg (-50%)	0.75 pg/mg (-25%)	1.25 pg/mg (+25%)	1.5 pg/mg (+50%)	1.75 pg/mg (+75%)	2.0 pg/mg (+100%)
Mean Abs. (450 nm)	2.028	1.839	1.621	1.452	1.133	1.001	0.915	0.869
S.D.	0.08143	0.05832	0.05005	0.06416	0.02243	0.01421	0.02788	0.03985
%CV	4.0	3.2	3.1	4.4	2.0	1.4	3.0	4.6

- THCA – ELISA Inter-Assay Precision using Spiked Samples (normalized data)

THCA Spiked Sample (n=200)	negative	0.25 pg/mg (-75%)	0.5 pg/mg (-50%)	0.75 pg/mg (-25%)	1.25 pg/mg (+25%)	1.5 pg/mg (+50%)	1.75 pg/mg (+75%)	2.0 pg/mg (+100%)
Mean Abs. (450 nm)	2.029	1.792	1.567	1.346	1.025	0.883	0.805	0.748
S.D.	0.08040	0.09879	0.10232	0.10455	0.09535	0.11120	0.09114	0.09029
%CV	4.0	5.5	6.5	7.8	9.3	12.6	11.3	12.1

# Omega study submitted for FDA 510(k) clearances

- THCA – ELISA Summary of Agreement Study Results

ELISA Test Result (n=422)	Negative by GC/MS (less than 50 pg/mg)	Less than half the cutoff concentration by GC/MS	Near Cutoff Negative (Between 50% below the cutoff and the cutoff concentration)	Near Cutoff Positive (Between the cutoff and 50% above the cutoff concentration)	High Positive (Greater than 50% above the cutoff concentration)
Positive	0	0	8	47	210
Negative	101	16	28	12	0

- THCA – ELISA Summary of Agreement Study Results

ELISA Test Result (n=422)	Negative by GC/MS (less than 50 pg/mg)	Less than half the cutoff concentration by GC/MS	Near Cutoff Negative (Between 50% below the cutoff and the cutoff concentration)	Near Cutoff Positive (Between the cutoff and 50% above the cutoff concentration)	High Positive (Greater than 50% above the cutoff concentration)
Positive	0	0	1.9%	11.1%	49.8%
Negative	23.9%	3.8%	6.6%	2.8%	0

# Omega study submitted for FDA 510(k) clearances

- Oxycodone – ELISA Intra-Assay Precision using Spiked Samples (non-normalized data)

Oxycodone Spiked Sample (n=11)	negative	75 pg/mg (-75%)	150 pg/mg (-50%)	225 pg/mg (-25%)	375 pg/mg (+25%)	450 pg/mg (+50%)	525 pg/mg (+75%)	600 pg/mg (+100%)
Mean Abs. (450 nm)	1.398	0.781	0.624	0.507	0.379	0.341	0.315	0.300
S.D.	0.06556	0.04891	0.02910	0.01944	0.01751	0.01461	0.01445	0.01435
%CV	4.7	6.3	4.7	3.8	4.6	4.3	4.6	4.8

- Oxycodone – ELISA Inter-Assay Precision using Spiked Samples (normalized data)

Oxycodone Spiked Sample (n=220)	negative	75 pg/mg (-75%)	150 pg/mg (-50%)	225 pg/mg (-25%)	375 pg/mg (+25%)	450 pg/mg (+50%)	525 pg/mg (+75%)	600 pg/mg (+100%)
Mean Abs. (450 nm)	1.398	0.796	0.636	0.520	0.386	0.343	0.310	0.302
S.D.	0.05907	0.04045	0.03628	0.04075	0.03347	0.03216	0.03098	0.02884
%CV	4.22	5.08	5.70	7.84	8.67	9.37	9.99	9.53

# Omega study submitted for FDA 510(k) clearances

- Oxycodone – ELISA Summary of Agreement Study Results

ELISA Test Result (n=422)	Negative by GC/MS (less than 50 pg/mg)	Less than half the cutoff concentration by GC/MS	Near Cutoff Negative (Between 50% below the cutoff and the cutoff concentration)	Near Cutoff Positive (Between the cutoff and 50% above the cutoff concentration)	High Positive (Greater than 50% above the cutoff concentration)
Positive	0	0	8	47	210
Negative	101	16	28	12	0

- Oxycodone – ELISA Summary of Agreement Study Results

ELISA Test Result (n=240)	Negative by GC/MS (less than 50 pg/mg)	Less than half the cutoff concentration by GC/MS	Near Cutoff Negative (Between 50% below the cutoff and the cutoff concentration)	Near Cutoff Positive (Between the cutoff and 50% above the cutoff concentration)	High Positive (Greater than 50% above the cutoff concentration)
Positive	0	0.8%	1.7%	3.3%	34.2%
Negative	50%	6.2%	2.5%	1.3%	0

# Sample Preparation Techniques

- Buffers or Organic Solvents
  - Whole hair/cut hair/powdered hair
- Acid or alkaline Hydrolysis
  - Not appropriate for all drug classes
- Enzymatic Digestion
- PT Programs have demonstrated that all techniques have the ability to work and provide accurate results

# Impact of Adulterants on ELISA

- “Toxin removal” Shampoos
  - Independent studies demonstrate ineffectiveness
  - Omega studies submitted for FDA 510(k) clearances

<b>ELISA Drug Panel</b>	<b>% POS after Treatment</b>	<b>% Change (GC/MS)</b>
Cocaine	100 (n=19)	-1%
Amphetamines	100 (n=23)	+5%
Opiates	94 (n=32)	-5%
PCP	100 (n=17)	-4%
THCA	85 (n=24)	-1%
Oxycodone	80 (n=10)	-18%

# Impact of Hair Treatments on ELISA

- Hygienic Treatments (Bleaching, Permanents, Dyes, Relaxers)
  - Omega studies submitted for FDA 510(k) clearances
  - Insignificant effect on negative specimens
  - Effect on Positive specimens within Standard Uncertainty - GC/MS confirmation assay

# Impact of Hair Treatments on ELISA (Continued)

- Hygienic Treatments - Bleaching

<b>ELISA Drug Panel</b>	<b>% POS after Treatment</b>	<b>% Change (GC/MS)</b>
Cocaine	96 (n=24)	-14%
Amphetamines	79 (n=24)	-11%
Opiates	100 (n=26)	-13%
PCP	100 (n=18)	-28%
THCA	88 (n=25)	-14%
Oxycodone	100 (n=6)	-12%

# Impact of Hair Treatments on ELISA (Continued)

- Hygienic Treatments - Permanents

<b>ELISA Drug Panel</b>	<b>% POS after Treatment</b>	<b>% Change (GC/MS)</b>
Cocaine	86 (n=22)	-12%
Amphetamines	92 (n=26)	-14%
Opiates	100 (n=23)	-11%
PCP	83 (n=18)	-36%
THCA	83 (n=24)	-12%
Oxycodone	100 (n=8)	-13%

# Impact of Adulterants on ELISA (Continued)

- Hygienic Treatments - Dyes

<b>ELISA Drug Panel</b>	<b>% POS after Treatment</b>	<b>% Change (GC/MS)</b>
Cocaine	100 (n=23)	-8%
Amphetamines	86 (n=21)	-8%
Opiates	100 (n=23)	-8%
PCP	89 (n=18)	-5%
THCA	80 (n=20)	-8%
Oxycodone	100 (n=5)	-19%

# Impact of Hair Treatments on ELISA (Continued)

- Hygienic Treatments - Relaxers

<b>ELISA Drug Panel</b>	<b>% POS after Treatment</b>	<b>% Change (GC/MS)</b>
Cocaine	100 (n=22)	-6%
Amphetamines	91 (n=22)	-8%
Opiates	100 (n=22)	-8%
PCP	94 (n=18)	-9%
THCA	67 (n=24)	-6%
Oxycodone	100 (n=8)	-20%

# Existing Hair Testing Standards

- Techniques scientifically accepted
- Hair Tests accepted in courts of law
- College of American Pathologists (CAP) FDT accreditation
  - Collection/sample handling
  - Extraction efficiencies
  - Wash/external contamination procedures
  - Quality Control
  - Required Proficiency Testing
- Accreditation to ISO/IEC 17025 standard
- European Workplace Drug Testing Guidelines
- United Nations Guidelines

# FDA Clearance of Laboratory Developed Screening Assays

- Omega Studies Required by FDA Included:
  - Agreement
  - Cosmetic Treatments
  - External Contamination
  - Precision
  - Recovery/Extraction Efficiency
  - Shipping Stability
  - Long Term Stability
  - Cross-reactivity
  - Detection Limits – ELISA and GC/MS
  - Traceability

# Proficiency Testing Validates Different Initial Testing Methodologies

- Sample Preparation
- Recovery/Extraction Efficiency
- Precision
- Cross-reactivity –  
opiates/amphetamines
- Detection Limits

QUESTIONS?