



Initial Testing for Drugs of Abuse in Hair Specimens

Prepared for

DTAB

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Presentation Overview

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

Cut-off levels (pg/mg)*

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

* 1pg = 1,000ng

Carboxy-THC** vs. THC***

Need for sensitive screening methods

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

Current laboratories with FDA cleared screening assays

Drug Panel	Omega	Quest	Psychemedics
Cocaine	500	300	500
Opiates	300	500	200
Amphetamines	500	300	500
PCP	300	300	300
Marijuana	1	1	1

Target Analytes

Drug Panel	Target Analyte
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	250 pg/mg	375 pg/mg	625 pg/mg	750 pg/mg	875 pg/mg	1000 pg/mg
		-75%	-50%	-25%	+25%	+50%	+75%	+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	250 pg/mg	375 pg/mg	625 pg/mg	750 pg/mg	875 pg/mg	1000 pg/mg
		-75%	-50%	-25%	+25%	+50%	+75%	+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)	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%	9.0%	6.7%	47.8%
Negative	35.3%	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	0.5 pg/mg	0.75 pg/mg	1.25 pg/mg	1.5 pg/mg	1.75 pg/mg	2.0 pg/mg
		-75%	-50%	-25%	+25%	+50%	+75%	+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	0.5 pg/mg	0.75 pg/mg	1.25 pg/mg	1.5 pg/mg	1.75 pg/mg	2.0 pg/mg
		-75%	-50%	-25%	+25%	+50%	+75%	+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=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	2	4	8	82
Negative	120	15	6	3	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

ELISA Drug Panel	% POS after Treatment	% Change (GC/MS)
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

ELISA Drug Panel	% POS after Treatment	% Change (GC/MS)
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

ELISA Drug Panel	% POS after Treatment	% Change (GC/MS)
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

ELISA Drug Panel	% POS after Treatment	% Change (GC/MS)
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

ELISA Drug Panel	% POS after Treatment	% Change (GC/MS)
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?