Detection of Trace Naltrexone and 6β-Naltrexol in Human Hair Using Enzyme Linked Immunosorbent Assay (ELISA)

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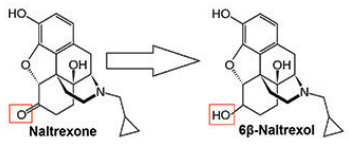
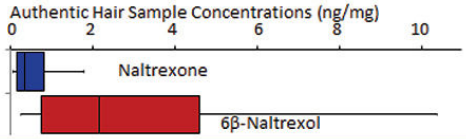
Objective

- Our laboratory has had a LC-MS/MS method for detection and quantification of naltrexone and 6β-naltrexol.
- However, one single method is not ideal in a forensic drug testing setting which mandates both initial and confirmatory testing of different methodologies
- Therefore, an initial detection method using ELISA was sought.

INTRODUCTION

- Ideal screening method must detect both naltrexone and 6β-naltrexol.[1-2]
- Cut-off is 100 pg/mg for LC-MS/MS.
- 6β-Naltrexol nearly always exists at a higher concentration than naltrexone in hair.





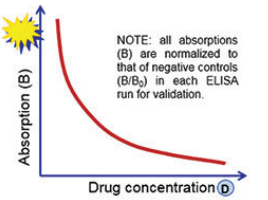
METHOD

- Sample Preparation

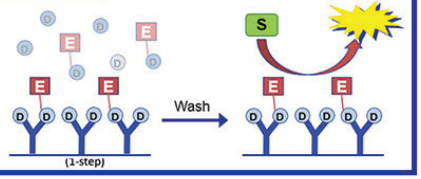
Aliquot
- 20 mg of 1.5 inch hair strands
- Acetone Wash
- Powdered

Extract
- 1.5 mL methanol
- 2-hour sonication with heat
- Centrifuge

ELISA
- Evaporate 1.0 mL methanol extract
- Reconstitute
- Heterogeneous-competitive ELISA

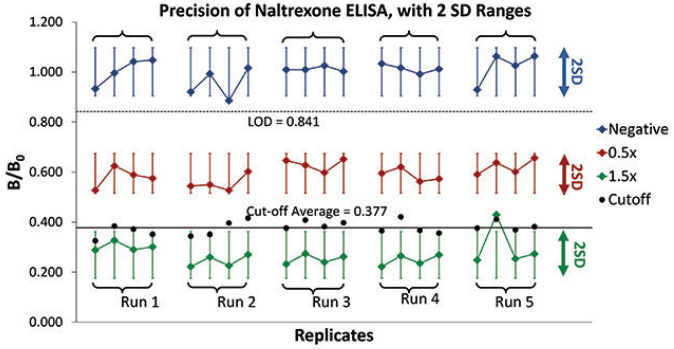


- Heterogeneous-Competitive ELISA (Immunanalysis®)

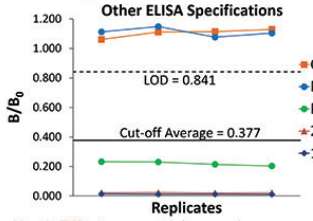


Results: Method Validation

- 5 runs each consisting of 4 controls at concentrations of 0.5x cut-off (50 pg/mg), 1.5x cut-off (150 pg/mg), and at the 100 pg/mg cut-off (Calibrator). Also, 4 negative controls were included in each run.

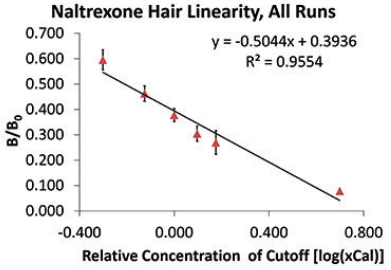


- All controls 0.5x – 100x cut-off had lower B/B_0 than the LOD.
- mean B/B_0 ± 2*SD for both 0.5x and 1.5x cut-off did not overlap the mean cut-off



- **Within-run:** CV% for controls 0.5x – 5x cut-off (50 – 500 pg/mg) = 3.72 – 10.5% not including run 5's outlier (28.61% with run 5's outlier)
- **Between-run:** CV% for controls 0.5x – 5x cut-off (50 – 500 pg/mg) = 4.7 – 10.9% not including run 5's outlier (17.3% with run 5's outlier)

- **Hook Effect** was not observed at 2.5 or 10 ng/mg
- **Carry-over** was not detected at concentrations of at least 10 ng/mg
- The ELISA showed good logarithmic correlation from concentrations of 50 to 500 pg/mg
- **Interference** from over the counter and prescription drugs at concentrations of 25 ng/mg was found negligible. (ephedrine, pseudo-ephedrine, phentermine, phenylpropanolamine, dihydrocodeine, ibuprofen, naproxen, ketoprofen, lidocaine, dextromethorphan)



Results: Authentic Hair Samples

- Authentic hair sample grouped based on LC-MS/MS results.

	Naltrexone (pg/mg)	6β-Naltrexol (pg/mg)	ELISA Absorbance	Qualitative Results
Calibrator at Cut-off	100	100	0.728	
<100 pg/mg Naltrexone Group	35	1181	0.212	POS
	72	502	0.166	POS
	90	2218	0.104	POS
Between 100 and 200 pg/mg Naltrexone	126	4009	0.115	POS
	154	877	0.174	POS
>200 pg/mg Naltrexone Group	369	9567	0.097	POS
	417	5804	0.83	POS
	437	2262	0.148	POS
	499	599	0.114	POS
	776	806	0.08	POS
	7977	6688	0.085	POS
	2150	3731	0.044	POS

DISCUSSION & CONCLUSION

- Majority of hair samples have 6β-naltrexol as the predominant form of naltrexone exposure.
- Immunanalysis® Naltrexone ELISA kit was validated according to SWGTOX guideline[3] to test hair samples as a screening method, and it gave satisfactory test sensitivity and specificity.
- The ELISA cross-reacted with 6β-naltrexol sufficiently, meaning the ELISA may test positive even when naltrexone is below cut-off in a hair sample.
- This satisfies our needs for two different test methodologies (ELISA and LC-MS/MS) to provide forensically defensible toxicology results.

References:

1) Heinala P, et al. Analysis of naltrexone and its metabolite 6-beta-naltrexol in serum with high-performance liquid chromatography. BMC Res Notes. 2012. 5:439.
2) McCaul, ME, et al. Serum 6-beta-naltrexol levels are related to alcohol responses in heavy drinkers. Alcoholism: Clinical and Experimental Research. 2000. 24(9): 1385-1391.
3) SWGTOX standard practices for method validation in forensic toxicology, J. Analytical Toxicology. 2013, 37, 452.

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