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.
- 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
  - 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 (Immunalysis®)

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.
- Replicates
- All controls 0.5x – 100x cut-off had lower B/B0 than the LOD.
- mean B/B0 ± 2 SD for both 0.5x and 1.5x cut-off did not overlap the mean cut-off.

Results: Authentic Hair Samples
- Authentic hair sample grouped based on LC-MS/MS results.

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 guideline1 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:
3) SWGTOX standard practices for method validation in forensic toxicology. J. Analytical Toxicology. 2013, 37, 482.

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