An Evaluation of the Immunalysis Buprenorphine Direct ELISA Kits for the Detection of Buprenorphines in Umbilical Cord

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OBJECTIVE

The objective of this study is to present a validated assay for the detection of buprenorphine in umbilical cord using Immunalysis Buprenorphine Direct ELISA kit.

BACKGROUND

- There has been an increase in maternal prescription opiate use resulting in an increase of treatment using buprenorphine.
- Infants exposed *in utero* to buprenorphine (BUP) may display neonatal abstinence syndrome (NAS) shortly after birth. [2]
- Therefore neonatal professionals need tools that are rapid, cost effective, and sensitive to identify newborns to refer them to appropriate treatment.

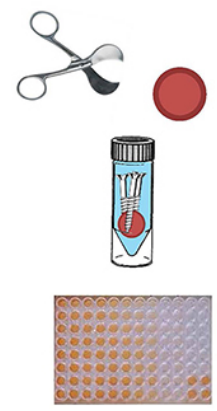
METHOD

- Sample Preparation

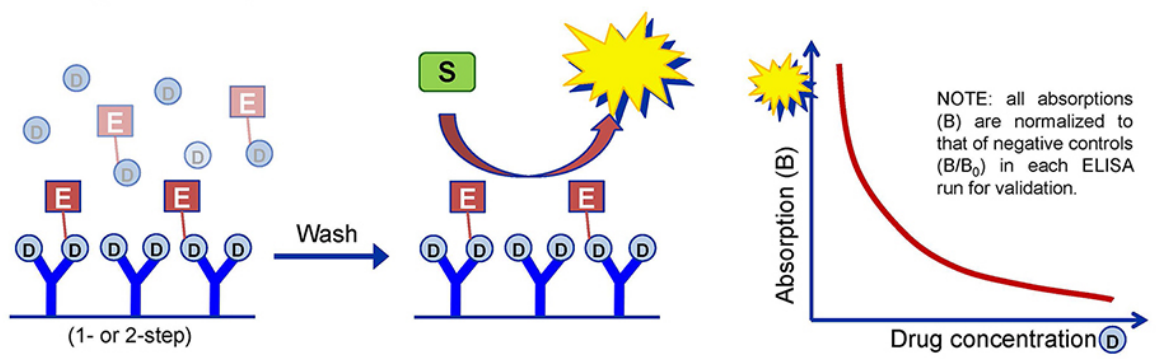
Aliquot
- 0.5 g
- Add Acetone

Extract
- BulletBlender®
- Centrifuge, decant, filter
- Dry (succinic acid)

ELISA
- Reconstitute 700 µL
- Multiplex ELISA



- Heterogeneous-Competitive ELISA



- Validation:
  - According to Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines.[1]
  - Controls prepared at 0.25 ng/g, 0.375 ng/g, 0.5 ng/g, 0.625 ng/g, and 0.75 ng/g to evaluate precision and accuracy.
  - Limit of detection (LOD) is determined by statistical signals in negative controls.
  - Controls prepared at 25x and 100x Cut-off to examine hook effect and carry-over.
  - Interferents: ephedrine, pseudoephedrine, phenylpropanolamine, phentermine, dihydrocodeine, ibuprofen, naproxen, ketoprofen, lidocaine, and dextromethorphan at 1000 ng/g.

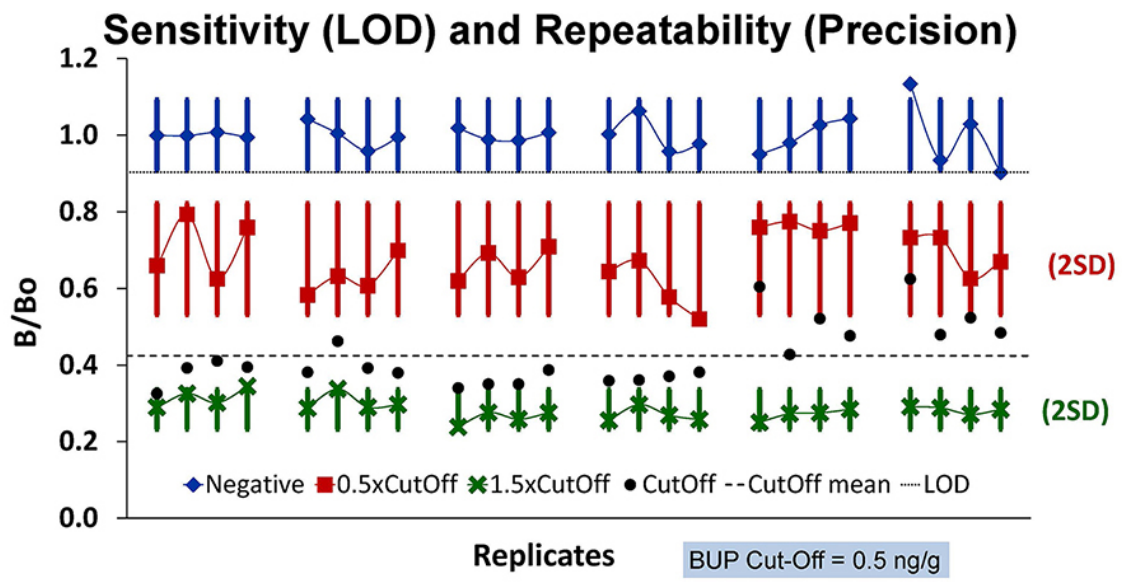
Schematic Representations:

Polyclonal antibody fixed in ELISA wells | D Drug to be analyzed | E–D Enzyme-linked drug conjugate | S Substrate to the drug-conjugated enzyme

CONCLUSION

The recent increase of prescription opiate abuse has resulted in an increase of maternal buprenorphine use. The method presented here demonstrates that the Immunalysis Buprenorphine Direct ELISA Kit may be utilized as an initial test for the detection of *in utero* buprenorphine exposure.

RESULTS



- Acceptable LOD criterion [LOD B/B_0 = Mean B_0 – 3.3 SD (dotted lines)]: LOD B/B_0 > all Cut-off B/B_0.
- Acceptable precision criteria: B/B_0 ± 2 SD ranges of **0.5x** and **1.5x** Cut-off do not overlap Cut-off B/B_0 mean (dashed lines); CV% of **0.5x – 1.5x Cut-off B/B_0** should be <20%.

ELISA Analytical Specification Summary

Drug	LOD (ng/g)	Within-Run CV% at 0.5-1.5x Cut-Off	Between-Run CV% at 0.5-1.5x Cut-Off	Confirmed + Rate*
BUP†	0.10	3.3-9.5%	9.0-19.7%	952/971 (98.0%)

†ELISA Cross-reacts with both buprenorphine and norbuprenorphine.
*971 samples were screened positive for buprenorphines by ELISA in 2013, and 952 were confirmed positive by the LC-MSMS method.

Sensitivity and Specificity

	LC-MSMS Positives	LC-MSMS Negatives
ELISA Positives	3	2
ELISA Negatives	0	23

Sensitivity=100% and Specificity=92%

- LC-MSMS method has LOQ of 0.4ng/g and LOD of 0.2ng/g for both buprenorphine and norbuprenorphine
- There were three true positve samples with the following quantitative values:
  Sample 1: 3.14 ng/g buprenorphine; 6.16 ng/g norbuprenorphine;
  Sample 2: 1.17 ng/g buprenorphine; 2.83 ng/g norbuprenorphine;
  Sample 3: 1.17 ng/g buprenorphine; 4.55 ng/g norbuprenorphine.

Assay Specificity

- Hook Effect/Carry-over:
  Hook Effect and Carry-over were not observed at least at 100x Cut-off of each drug class for all ELISAs.
- Interferents:
  The ELISAs showed negligible B/B_0 signals from the tested over-the-counter or prescription drugs.

REFERENCES

1) SWGTOX, J. Anal. Toxicol. 2013, 37, 452.
2) Department of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA