Comparison of oral fluid proficiency specimens utilizing homogeneous enzyme immunoassay (HEIA) and ELISA

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Abstract

• Proficiency samples are used to challenge both screening and confirmatory laboratory procedures.

• With the increased sensitivity of rapid, simple high throughput screening platforms using liquid reagents, homogeneous enzyme immunoassay (HEIA) has begun to increase in popularity as a screening choice for oral fluid, which to date has been carried out using enzyme linked immunosorbent assays (ELISA).

Objective

• To determine whether HEIA proficiency data correlates well to ELISA data over a span of 5 testing cycles.

Methods

• Neat oral fluid proficiency samples received from January 2008 to May 2009 were all reanalyzed in June 2009 by both HEIA and ELISA.

• Proficiency samples were stored at 4 C in their original amber glass containers and were not sampled again until June 2009.

• All samples (N=23) and calibrators were diluted 1+3 with Quantisal™ buffer to achieve concentration values recommended for their corresponding tests. Two proficiency specimens had no volume remaining.

• All samples were analyzed for the following drugs:

Drug Class	ELISA cut-off	HEIA cut-off
Cannabinoids (THC)	4ng/mL	8ng/mL
Cocaine (COC)	20ng/mL	20ng/mL
AMP / METH	50ng/mL	50ng/mL
Opiates (OPI)	40ng/mL	40ng/mL
Phencyclidine (PCP)	10ng/mL	10ng/mL
Benzodiazepines (BZP)	10ng/mL	10ng/mL
Methadone (MTD)	50ng/mL	50ng/mL
Oxycodone (OXYC)	25ng/mL	40ng/mL

Results

• For OPI, PCP, MTD, and OXYC all 23 samples had 100% correlation (POS/NEG).

• METH showed a correlation of 95%; one sample screening negatively using HEIA was found to have 48ng/mL of methamphetamine, just below the cut off concentration.

• The AMP correlation was 91% with HEIA detecting two samples containing only MDA, though minor inhibition was seen in the raw data. In this case there are different antibodies used and the cross-reactivity for MDA differs between the two kits by 178% for ELISA and 40% for HEIA.

• The BZP correlation was 91% with one sample containing 11 ng/mL oxazepam and the second sample containing 2.1 ng/mL alprazolam.

• The lowest correlation, as expected, was in the drug classes COC and THC (86% correlation). For THC the samples that did not agree all challenged the cut-off concentrations for both kits with three samples screening positive by ELISA and negative by HEIA.

• As with the amphetamine antibody the cocaine HEIA and ELISA antibodies do differ slightly with respect to cross-reactivity. One more was found positive by ELISA but negative with HEIA.

• In all cases where POS/NEG data did not correlate inhibition was seen in the raw data for HEIA and ELISA within +/- 20% of cut-off concentration.

Data

ID	DRUG CLASS	ELISA 2009	HEIA 2009	GC 2009 (ng/mL)	Possible Cause
2008 OFU-10	THC	POS	NEG	9.5	Low level inhibition seen in HEIA
2009 OFU-04	THC	POS	NEG	7.6	Low level inhibition seen in HEIA
2009 OFU-08	THC	POS	NEG	4.9	below cut-off
2008 OFU-07	COC	POS	NEG	24	Low level inhibition seen in HEIA
2008 OFU-07	AMP	POS	NEG	75 (MDA)	antibody
2009 OFU-06	AMP	POS	NEG	88 (MDA)	antibody
2009 OFU-01	METH	POS	NEG	48	Low level inhibition seen in HEIA
2008 OFU-03	BZP	POS	NEG	11 (OXAZ)	Low level inhibition seen in HEIA
2008 OFU-12	BZP	POS	NEG	2.1 (ALP)	low level

Summary

• The overall correlation between HEIA and ELISA using proficiency samples was 94%.

• Routine liquid reagent chemistry analyzers and ELISA platforms show a high degree of qualitative correlation.

• Discrepant results can be attributed to different cut-off concentrations, or variation in the antibodies used in the assays.

Oral Fluid

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