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).

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 Quansat™ 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:

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>ELISA cut-off</th>
<th>HEIA cut-off</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cannabinoids (THC)</td>
<td>4ng/mL</td>
<td>8ng/mL</td>
</tr>
<tr>
<td>Cocaine (COC)</td>
<td>20ng/mL</td>
<td>20ng/mL</td>
</tr>
<tr>
<td>AMP / METH</td>
<td>50ng/mL</td>
<td>50ng/mL</td>
</tr>
<tr>
<td>Opiates (OPI)</td>
<td>40ng/mL</td>
<td>40ng/mL</td>
</tr>
<tr>
<td>Phencyclidine (PCP)</td>
<td>10ng/mL</td>
<td>10ng/mL</td>
</tr>
<tr>
<td>Benzodiazepines (BZP)</td>
<td>10ng/mL</td>
<td>10ng/mL</td>
</tr>
<tr>
<td>Methadone (MTD)</td>
<td>50ng/mL</td>
<td>50ng/mL</td>
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<tr>
<td>Oxycodeine (OXYC)</td>
<td>25ng/mL</td>
<td>40ng/mL</td>
</tr>
</tbody>
</table>

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 AMF 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 11ng/mL oxazepam and the second sample containing 2.1ng/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.

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.

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 |
2008 OFU-08 | COC | POS | NEG | 24 | Low level inhibition seen in HEIA |
2009 OFU-07 | AMP | POS | NEG | 75 (MDA) | antibody |
2008 OFU-07 | AMP | POS | NEG | 86 (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 |
2008 OFU-12 | BZP | POS | NEG | 2.1 (ALP) | low level |

Objective

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