The Comparison of a New Chemiluminescence Immunoassay Screen with a Single-Step Enzyle Linked Imunosorbent Assay (ELISA)

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AIMS: The current procedures used by the Toxicology Laboratory, Forensic Science South Australia, for the screening of whole blood (postmortem and antemortem) for drugs of abuse (opiates, benzodiazepines, cannabinoids and amphetamines) is a single-step ELISA screen followed by either GC-MS or LC-MS/MS for confirmation of the parent drug. Randox has recently released a new chemiluminescence immunoassay system. The principle used in this immunoassay technique is similar to other heterogeneous assays but offers greater sensitivity using chemiluminescence. The aim of this report is to compare this technique to the existing ELISA technique.

METHODS: The Toxicology Laboratory has conducted an evaluation of this new technique by comparison to results obtained from 185 whole blood samples (145 post-mortem and 40 ante-mortem) using our routine screening system (ELISA) with MS confirmation. The cut-off concentrations for the Randox Evidence drugs of abuse (DoA) assay were set at the following: opiates (10 ng/mL), benzodiazepines (10 ng/mL), carboxy-THC (5 ng/mL) and methamphetamine (20 ng/mL). All samples were subjected to our standard confirmation methods to verify the immunoassay results. Opiates (morphine, codeine and 6-monoacetylmorphine) were analysed by solid phase extraction (SPE) followed by LC-MS/MS. Benzodiazepines were confirmed by liquid-liquid extraction followed by capillary GC-MS (thermally unstable benzodiazepines e.g. temazepam and oxazepam were confirmed by LC-MS/MS). Amphetamines (methamphetamine, amphetamine, MDMA, MDA), THC and carboxy-THC were confirmed by SPE followed by derivatisation and capillary GC-MS.

RESULTS: The following table summarises the results obtained:

<table>
<thead>
<tr>
<th></th>
<th>Amphetamines</th>
<th>Opiates</th>
<th>Benzodiazepines</th>
<th>Cannabinoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>True Positives (TP)</td>
<td>14.5%</td>
<td>20%</td>
<td>22.7%</td>
<td>14.5%</td>
</tr>
<tr>
<td>False Positives (FP)</td>
<td>40%</td>
<td>6.4%</td>
<td>2.7%</td>
<td>4.8%</td>
</tr>
<tr>
<td>False Negatives (FN)</td>
<td>10.8%</td>
<td>1%</td>
<td>1.1%</td>
<td>ND(^1)</td>
</tr>
<tr>
<td>True Negatives (TN)</td>
<td>35.1%</td>
<td>71.3%</td>
<td>68.6%</td>
<td>77.3%</td>
</tr>
</tbody>
</table>

Sensitivity: 0.57 0.94 0.95 ND\(^4\)
Specificity: 0.49 0.91 0.96 0.94
Positive Predictive Value (PPV): 0.28 0.75 0.89 0.75
Negative Predictive Value (NPV): 0.76 0.98 0.98 ND\(^4\)

\(^1\) Six (6) samples were putrefactive effusions from decomposed bodies
\(^2\) Eight (8) samples contained MDMA
\(^3\) Antemortem samples only analysed for THC, may be inflated, as Carboxy-THC may be present
\(^4\) ND - Not determined as laboratory analysis is not complete at this time

Keywords: Chemiluminescence, Drugs of abuse, Immunoassay