OPTIMISATION OF THE BIOCHIP ARRAY DOA ULTRA FOR ENHANCED MULTIPLEX DETECTION OF DRUGS RELATED TO IMPAIRED DRIVING

L. Keery, C. Stevenson, F. O'Muilleoir, A. Brown, J. Darragh, M.L. Rodríguez, R.I. McConnell, S.P. FitzGerald
Randox Toxicology Ltd. Crumlin, United Kingdom
e-mail: scientific.publications@randox.com

Introduction

Drug detection involves initial screening of samples for drugs. Drug impaired driving is becoming a major problem worldwide. Biochip array technology allows the simultaneous screening of multiple drugs related to impaired driving from a single undivided sample of blood or urine.

Biochip-based platform DoA Ultra Array → simultaneous detection:				
Amphetamine	Barbiturates	Benzodiazepines	Benzoylecgonine/cocaine	Buprenorphine
Cannabinoids	Dextromethorphan	Fentanyl	Generic opioids	Meprobamate
Methadone	Methamphetamine	Opiates	Oxycodone	Phencyclidine
Tramadol	Tricyclic antidepressants	Zolpidem	-	-
→ including parent compounds and metabolites				

The optimal analytical performance of this array was previously reported (SOFT 2015, 2016).
Updated (2017) recommendations for the toxicological investigation of suspected alcohol and drug -impaired driving cases and motor vehicle fatalities have been reported[1].

In view of these recommendations, this study aimed to **optimise the DoA Ultra Array** for enhanced multiplex detection:

- A new assay was introduced for the detection of **clonazepam.**
- The specificity profile of the assay standardised to oxazepam was expanded to detect **oxazepam and temazepam glucuronides**.
- The screening **cut-offs** of the **buprenorphine** (blood) and **fentanyl** (blood and urine) assays were reduced.

Methodology

Simultaneous competitive chemiluminescent immunoassays, defining discrete test sites on the biochip surface, were employed. The assays were applied to the Evidence series analysers. The light signal generated from each of the test sites on the biochip was detected using digital imaging technology and compared to that from a stored calibration curve. The signal output is inversely proportional to the concentration of drug in the sample. The systems have dedicated software to process, report and archive the data produced. Sample volume is 10µl (neat urine) and 60µl of 1 in 4 diluted blood.

Results

Results focused on the new and optimised assays are summarised.

New assay standardised to clonazepam

The addition on the biochip surface of the immunoassay standardised to clonazepam allowed detection of this drug as well as 7 amino clonazepam (cross-reactivity 40%).

New assay standardised to clonazepam cut-offs and LODs:

Matrix	Cut-Off	LOD
Blood	10 ng/mL	<1 ng/mL
Urine	25 ng/mL	<1 ng/mL

Assay standardised to oxazepam

The assay standardised to oxazepam detected other 17 compounds with cross-reactivity >25%.

Compund	Cross-reactivity (%)
Oxazepam	100
Alprazolam	252.2
Nordiazepam	187.1
Estazolam	128.7
Midazolam	109.2
Oxazepam glucuronide	100.5
Diazepam	100
Bromazepam	97.1
Clobazam	75.1
Temazepam	69.1
Desalkylflunitrazepam	54.4
α- OH-Alprazolam	51.7
Nitrazepam	49.7
N-Desmethylflunitrazepam	35.2
Phenazepam	34.8
Triazolam	26.8
Lorazepam	25.7
Temazepam glucuronide	25.7
Lorazepam glucuronide	15.5

Assay standardised to oxazepam cut-offs and LODs:

Matrix	Cut-Off	LOD
Blood	10 ng/mL	5 ng/mL
Urine	100 ng/mL	20 ng/mL

Reduction of screening cut-offs buprenorphine assay (blood) and fentanyl assay (blood and urine)

Buprenorphine assay and fentanyl assay cut-offs and LODs

Assay	Matrix	Cut-Off	LOD
Buprenorphine	Blood	1 ng/mL	<0.2 ng/mL
Fentanyl	Blood	1 ng/mL	< 0.2 ng/mL
	Urine	1 ng/mL	< 0.2 ng/mL

Overall precision, expressed as CV(%), was <20%.

19.024.15 | RDFT, 19.013.159RDFT, 17.063.140RDFT, 17.005.142RDFT

Conclusion

Considering the updated (2017) recommendation for the toxicological investigation of suspected alcohol and drug -impaired driving cases and motor vehicle fatalities, the flexibility of biochip array technology allowed the inclusion of a new assay in the pre-existent DoA Ultra Array for the detection of the Tier 1 compound clonazepam. Moreover, the specificity of one assay standardised to oxazepam was expanded to include detection of glucuronides, which is relevant when screening urine samples. The cut-offs of the buprenorphine (blood) and fentanyl (urine and blood) assays were reduced to 1ng/mL.
In total, with this optimised biochip array >100 compounds can be detected with cross-reactivity >20% including parent compounds and metabolites. This application allows the simultaneous screening of a broad range of drugs related to impaired driving from a single sample. The use of this platform increases the screening capacity in the drug testing process.

Reference

Logan B.K., *et al.* Recommendations for Toxicological Investigation of Drug-Impaired Driving and Motor Vehicle Fatalities-2017 Update. *J Anal Toxicol.* 2018, 42(2): 63-68