CONSOLIDATION AND EFFICIENCY IN TOXICOLOGY WITH BIOCHIP ARRAY TECHNOLOGY APPLIED TO DUID TESTING

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Introduction

For the detection of drugs in biological samples, an initial screening is performed to eliminate all negative results; positive results require confirmation using confirmatory methods. The availability of reliable screening methods enabling the detection of multiple drugs consolidates the testing process and leads to laboratory efficiency. Biochip array technology enables the screening of multiple drugs from a single sample.

This study reports the DOA ULTRA/DUID biochip array containing 20 immunoassays for the simultaneous screening of drugs of abuse in urine and whole blood, which represents the most extensive multi-analytical screening approach suitable for Driving Under the Influence of Drugs Testing (DUID).

Methodology

Multiple miniaturized competitive chemiluminescent immunoassays, arrayed on a biochip defining discrete test sites, are applied to dedicated biochip analysers: Evidence, Evidence Investigator and Evidence Evolution. These systems incorporate dedicated software to process, report and archive the multiple data produced.

Test menu

Amphetamine	Methamphetamine
Barbiturates	Meprobamate
Benzodiazepine 1	Methadone
Benzodiazepine 2	Opiates
Buprenorphine	Oxycodone 1
Cannabinoids	Oxycodone 2
Cocaine metabolite (benzoylecgonine)/Cocaine	Phencyclidine
Dextromethorphan	Tramadol
Fentanyl	Tricyclic antidepressants (TCAs generic)
Generic opioids*	Zolpidem
Ketamine**	

* Evidence and Evidence Investigator only **Evidence Evolution only

Biochip Array Technology

Biochip
(9mm x 9mm)

Discrete
Test Regions

Evidence
Fully automated, floor standing biochip analyser

Evidence Evolution
Fully automated, random access floor standing biochip analyser

Evidence Investigator
Semi-automated benchtop biochip analyser

Urine samples are used neat, 6µL are required for the Evidence biochip analyser and 10µL for Evidence Evolution and Evidence Investigator. Whole blood samples are ready to use after a simple dilution (1:4) and 60 µL are required.

Reference

1. Logan, B.K. *et al.* Recommendations for toxicological investigation of drug-impaired driving and motor vehicle fatalities. *J. Anal. Toxicol.* 2013:37(8):552-558.

Results

Specificity/Cross-Reactivity (CR) – Compounds CR (%) >20

Amphetamine Assay	Benzodiazepine 1 Assay	Benzodiazepine 2 Assay	Dextromethorphan Assay	Ketamine Assay**	Opiates Assay	Tramadol Assay	Zolpidem Assay
S(+) Amphetamine	Oxazepam	Lorazepam	Dextromethorphan	Ketamine	Morphine	Tramadol	Zolpidem
±MDA	Temazepam	Phenazepam	Dextrorphan tartrate salt	Norketamine	Hydrocodone	O-Desmethyltramadol	4-Carboxyzolpidem
PMA HCl	Nordiazepam	Clonazepam	(±)-Nordextromethorphan		Ethyl morphine HCl		
BDB	α-OH-alprazolam	Lorazepam glucuronide (blood)		Meprobamate Assay	Codeine	TCAs Assay	
±Amphetamine	Alprazolam		Generic Opioids Assay*	Meprobamate	6-Acetyl-codeine	Nortriptyline	
Phentermine	Diazepam	Buprenorphine Assay	Oxycodone	Carisoprodol	Hydromorphone	Imipramine N oxide	
5-IT	Estazolam	Norbuprenorphine (urine)	Morphine (urine)		Desomorphine	Imipramine	
5-APB HCl	Clobazam	Buprenorphine (blood)	Hydrocodone	Methadone Assay	Morphine-6BD-glucuronide	Trimipramine	
6-APB HCl	Nitrazepam	Buprenorphine-3B-D-glucuronide (blood)	Ethyl morphine HCl	Methadone	Heroin	Desipramine	
5-APDB HCl	2-OH-ethylflurazepam		Codeine		6-MAM	Cyclobenzaprine	
	Prazepam	Cannabinoids Assay	6-Acetyl-codeine	Methamphetamine Assay		Amitriptyline	
Barbituates Assay	Midazolam	11-nor-Δ9-THC-carboxylic acid (urine)	Dihydrocodeine	S(+)-Methamphetamine	Oxycodone 1 Assay	Opipramol	
Phenobarbital	Flunitrazepam	(-)-11- nor-9-Carboxy- Δ9-THC (blood)	Hydromorphone	PMMA HCl	Oxycodone	Promazine	
Secobarbital	Flurazepam	(±)-11-Hydroxy-Δ9-THC (blood)	Desomorphine	MDMA	Hydrocodone	Maprotiline	
Butabarbital	Phenazepam		Morphine-3BD-glucuronide (blood)	(±)-Methamphetamine	Noroxycodone	Doxepin	
Pentobarbital	Desalkylflunitrazepam	Cocaine Metabolite (BZG) Assay		5-MAPB HCl		Clomipramine	
Alphenal	Lormetazepam	Benzoylecgonine	Fentanyl Assay	5-MAPDB HCl	Oxycodone 2 Assay	Protryptiline	
Cyclopentobarbital	Chlordiazepoxide	Cocaine	Fentanyl		Oxycodone	Cyproheptadine	
p-OH-phenobarbital	Triazolam	m-Hydroxybenzoylecgonine	α-Methylfentanyl		Oxymorphone	Lofepramine	
Butalbital	Etizolam	Cocaethylene	p-Fluorofentanyl			Dothiepin	
Amobarbital	N-desmethylflunitrazepam		Benzylfentanyl		Phencyclidine Assay	Chlorpromazine	
Barbital	Bromazepam		Butyrylfentanyl HCl		Phencyclidine		
			Norfentanyl				

16.039-041.12ARDFT, 16.008.123RDFT

* Evidence and Evidence Investigator only **Evidence Evolution only

Cut-offs

Assay	Urine Cut-off (ng/mL)	Blood Cut-off (ng/mL)
Amphetamine	200***	20
Barbiturates	200	50
Benzodiazepine 1	100***	10
Benzodiazepine 2	100***	10
Buprenorphine	5	5
Cannabinoids	20***	10
Cocaine metabolite (Benzoylecgonine)	150***	50
Dextromethorphan	20	5
Generic Opioids*	100***	10
Fentanyl	2	2
Ketamine**	750	-

16.043.12ARDFT

Assay	Urine Cut-off (ng/mL)	Blood Cut-off (ng/mL)
Meprobamate	500***	100
Methadone	300***	10
Methamphetamine	200***	20
Opiates	200***	10
Oxycodone 1	100***	10
Oxycodone 2	100***	10
Phencyclidine	25***	5
Tramadol	5	5
Tricyclic antidepressants (TCAs generic)	100	60
Zolpidem	10	10

16.043.12ARDFT

* Evidence and Evidence Investigator only ** Evidence Evolution only *** Cut-offs as per DUID recommendations[1]

The cut-off values were further validated by assessing inter-assay precision. Samples were spiked with the appropriate drug compound 50% below, at the cut-off, and 50% above the recommended cut-off. Three replicates were assessed over 5 separate runs and the inter-assay precision calculated to be less than 20% for all levels across all assays.

Intra-assay precision was also assessed with 20 replicates of a low, mid and high concentration sample within one run; resultant intra-assay precision was also calculated to be less than 20% for all levels across all assays.

Conclusions

The results indicate applicability of the DOA ULTRA/DUID biochip array to the multi-drug screening in urine and blood, which leads to test consolidation and an increase in the screening capacity. This biochip array includes assays applicable to DUID testing and represents the most extensive screening tool for drugs in urine and blood samples.

The assays are applicable to a variety of dedicated biochip analysers (Evidence, Evidence Investigator, Evidence Evolution) which incorporate the software to process, report and archive the multiple data generated. By using this technology, the laboratory efficiency in the initial screening step of the drug testing process is increased.