All-Inclusive DUID Drug Screening through the Use of 20 Immunoassays Defining Discrete Test Sites on a New Biochip Array

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Introduction

- Drug detection involves initial screening of samples for drugs. Drug impaired driving is becoming a major problem worldwide and recommendations for toxicological investigation of drug-impaired driving and motor vehicle fatalities were reported. In these recommendations the most prevalent tier 1 drugs found in the US impaired driving population should be the minimum testing to be completed in drug driving casework. Tier 2 drugs are less frequently encountered. Recommended cut-offs suitable for urine and blood were stated.[1]
- This study reports the application of biochip array technology to the simultaneous screening of tier 1 and tier 2 drugs from a single sample of urine or blood by using simultaneous immunoassays, which define discrete test sites on the biochip surface.
- This multi-analytical approach results in an increase of the DUID (Driving Under the Influence of Drugs) screening capacity in tests settings and it facilitates the drug testing process. For legal purposes, the screening procedure eliminates all negatives, and positive results are regarded as presumptive and require confirmation using confirmatory methods.

Methodology

- Twenty competitive chemiluminescent biochip-based immunoassays were employed and applied to the Evidence biochip analyser.
- The signal output is inversely proportional to the concentration of drug in the sample.
- Two panels were developed so that the desired cut-offs were achieved in each matrix and that the relevant parent and metabolite compounds were detected in urine and the whole blood respectively.
- The system has dedicated software to process, report and archive the data produced. The sample volume required is 6 µl of neat urine and 60µl of whole blood (diluted 1 in 4).

DoA ULTRA/DUID	
Amphetamine	Meprobamate
Barbiturates	Methadone
Benzodiazepines I (Oxazepam)	Methamphetamine
Benzodiazepines II (Lorazepam)	Opiate
Benzoylecgonine (Cocaine Metabolite)	Oxycodone I
Buprenorphine	Oxycodone II
Cannabinoids (THC)	Phencyclidine (PCP)
Dextromethorphan	Tramadol
Fentanyl	Tricyclic Antidepressants (TCA)
Generic Opioids	Zolpidem

Biochip (9mm x 9mm)

Biochip carrier (3 x 3 biochips)

Fully automated Evidence analyser

Results

Specificity/ Cross-reactivity (CR)			
Amphetamine Assay Compounds CR (%) >20	Barbiturates assay Compounds CR (%) >20	Benzodiazepine 1 assay Compounds CR (%) >20	Benzodiazepine 2 assay Compounds CR (%) >20
S(+) Amphetamine	Phenobarbital	Oxazepam	Lorazepam
±MDA	Secobarbital	Temazepam	Phenazepam
PMA HCl	Butabarbital	Nordiazepam	Clonazepam
BDB	Pentobarbital	α-OH-alprazolam	Lorazepam glucuronide (blood)
±Amphetamine	Alphenal	Alprazolam	
Phentermine	Cyclopentobarbital	Diazepam	
5-IT	p-OH-phenobarbital	Estazolam	
5-APB HCl	Butalbital	Clobazam	
6-APB HCl	Amobarbital	Nitrazepam	
5-APDB HCl	Barbital	2-OH-ethylflurazepam	
		Prazepam	
		Midazolam	
		Flunitrazepam	
		Flurazepam	
		Phenazepam	
		Desalkylflunitrazepam	
		Lormetazepam	
		Chlordiazepoxide	
		Triazolam	
		Etizolam	
		N-desmethylflunitrazepam	
		Bromazepam	
Buprenorphine assay Compounds CR (%)>20	Cannabinoids assay Compounds CR (%)>20	Cocaine metabolite (BZG) assay Compounds CR (%)>20	Dextromethorphan assay Compounds CR (%)>20
Norbuprenorphine (urine)	11-nor-Δ9-THC-carboxylic acid (urine)	Benzoylecgonine	Dextromethorphan
Buprenorphine (blood)	(-)-11- nor-9-Carboxy- Δ9-THC (blood)	Cocaine	Dextrorphan tartrate salt
Buprenorphine-3B-D- glucuronide (blood)	(±)-11-Hydroxy-Δ9-THC (blood)	m-Hydroxybenzoylecgonine	(±)-Nordextromethorphan
		Cocaethylene	
Generic opioids assay Compounds CR (%)>20	Fentanyl assay Compounds CR (%)>20	Meprobamate assay Compounds CR (%)>20	Methadone assay Compounds CR (%)>20
Oxycodone	Fentanyl	Meprobamate	Methadone
Morphine (urine)	α-Methylfentanyl	Carisoprodol	
Hydrocodone	p-Fluorofentanyl		
Ethyl morphine HCl	Benzylfentanyl		
Codeine	Butyrylfentanyl HCl		
6-Acetyl-codeine	Norfentanyl		
Dihydrocodeine			
Hydromorphone			
Desomorphine			
Morphine-3BD-glucuronide (blood)			
Methamphetamine assay Compounds CR (%)>20	Opiates assay Compounds CR (%)>20	Oxycodone 1 assay Compounds CR (%)>20	Oxycodone 2 assay Compounds CR (%)>20
S(+)-methamphetamine	Morphine	Oxycodone	Oxycodone
PMMA HCl	Hydrocodone	Hydrocodone	Oxymorphone
MDMA	Ethyl morphine HCl	Noroxycodone	
(±)-Methamphetamine	Codeine		
5-MAPB HCl	6-Acetyl-codeine		
5-MAPDB HCl	Hydromorphone		
	Desomorphine		
	Morphine-6BD-glucuronide		
	Heroin		
	6-MAM		
Phencyclidine assay Compounds CR (%)>20	Tramadol assay Compounds CR (%)>20	TCAs assay Compounds CR (%)>20	Zolpidem assay Compounds CR (%)>20
Phencyclidine	Tramadol	Nortriptyline	Zolpidem
	O-Desmethyltramadol	Imipramine N oxide	4-Carboxyzolpidem
		Imipramine	
		Trimipramine	
		Desipramine	
		Cyclobenzaprine	
		Amitriptyline	
		Opipramol	
		Promazine	
		Maprotiline	
		Doxepin	
		Clomipramine	
		Protryptiline	
		Cyproheptadine	
		Lofepramine	
		Dothiepin	
		Chlorpromazine	

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Urine

Limits of Detection (LOD) and cut-offs		
Assay	LOD (ng/mL)	Cut-off (ng/mL)
Amphetamine	31.73	200*
Barbiturates	25.12	200
Benzodiazepine 1	0.77	100*
Benzodiazepine 2	2.37	100*
Buprenorphine	0.12	5
Cannabinoids	1.22	20*
Cocaine metabolite (Benzoylecgonine)	8.64	150*
Dextromethorphan	0.59	20
Generic Opioids	6.85	100*
Fentanyl	0.19	2
Meprobamate	9.56	500*
Methadone	4.67	300*
Methamphetamine	7.88	200*
Opiates	13.39	200*
Oxycodone 1	3.64	100*
Oxycodone 2	0.76	100*
Phencyclidine	0.87	25*
Tramadol	0.89	5
Tricyclic antidepressants (TCAs generic)	4.63	100
Zolpidem	0.48	10

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* Cut-offs as per DUID recommendations

Intra-assay precision			
Assay	Precision material 1 CV (%)	Precision material 2 CV (%)	Precision material 3 CV (%)
Amphetamine	6.3	7.6	18.5
Barbiturates	9.9	7.0	9.8
Benzodiazepine 1	13.7	10.4	12.6
Benzodiazepine 2	8.1	7.2	13.0
Buprenorphine	6.1	9.8	8.3
Cannabinoids	7.8	12.6	7.8
Cocaine metabolite (Benzoylecgonine)	10.8	9.0	9.2
Dextromethorphan	6.0	7.3	10.8
Generic Opioids	9.4	7.6	9.8
Fentanyl	15.2	14.1	18.3
Meprobamate	7.0	6.7	8.8
Methadone	5.1	8.6	7.4
Methamphetamine	5.7	3.9	4.8
Opiates	5.5	4.8	5.6
Oxycodone 1	14.5	11.3	8.9
Oxycodone 2	17.2	13.2	16.7
Phencyclidine	6.0	8.7	10.3
Tramadol	10.4	8.5	6.5
Tricyclic antidepressants (TCAs generic)	6.0	6.9	6.9
Zolpidem	7.4	11.3	13.3

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Blood

Limits of Detection (LOD) and cut-offs		
Assay	LOD (ng/mL)	Cut-off (ng/mL)
Amphetamine	2.76	20
Barbiturates	3.67	50
Benzodiazepine 1	0.21	10
Benzodiazepine 2	0.60	10
Buprenorphine	0.11	5
Cannabinoids	2.96	10
Cocaine metabolite (Benzoylecgonine)	1.03	50
Dextromethorphan	0.07	5
Generic Opioids	1.23	10
Fentanyl	0.09	2
Meprobamate	7.23	100
Methadone	1.46	10
Methamphetamine	10.0	20
Opiates	0.50	10
Oxycodone 1	1.01	10
Oxycodone 2	0.73	10
Phencyclidine	0.27	5
Tramadol	0.34	5
Tricyclic antidepressants (TCAs generic)	2.77	60
Zolpidem	0.35	10

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Intra-assay precision			
Assay	Precision material 1 CV (%)	Precision material 2 CV (%)	Precision material 3 CV (%)
Amphetamine	13	5	6
Barbiturates	5	6	5
Benzodiazepine 1	11	10	14
Benzodiazepine 2	7	7	9
Buprenorphine	12	11	12
Cannabinoids	5	11	14
Cocaine metabolite (Benzoylecgonine)	6	7	7
Dextromethorphan	13	7	9
Generic Opioids	15	9	14
Fentanyl	6	7	5
Meprobamate	12	7	7
Methadone	6	4	6
Methamphetamine	6	6	6
Opiates	11	13	9
Oxycodone 1	19	12	11
Oxycodone 2	8	10	14
Phencyclidine	10	6	4
Tramadol	8	5	4
Tricyclic antidepressants (TCAs generic)	6	4	5
Zolpidem	8	6	7

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Conclusion

The results indicate applicability of biochip array technology to the simultaneous screening of drugs associated with DUID in Tier 1 and Tier 2 under reported recommendations. The twenty immunoassays arrayed on each biochip surface presented both the desired sensitivity and reproducibility required to achieve screening at the recommended cut-offs. This methodology allows for multi-analytical screening of samples, leading to test consolidation and increased screening capacity in test settings.

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.