

Introduction

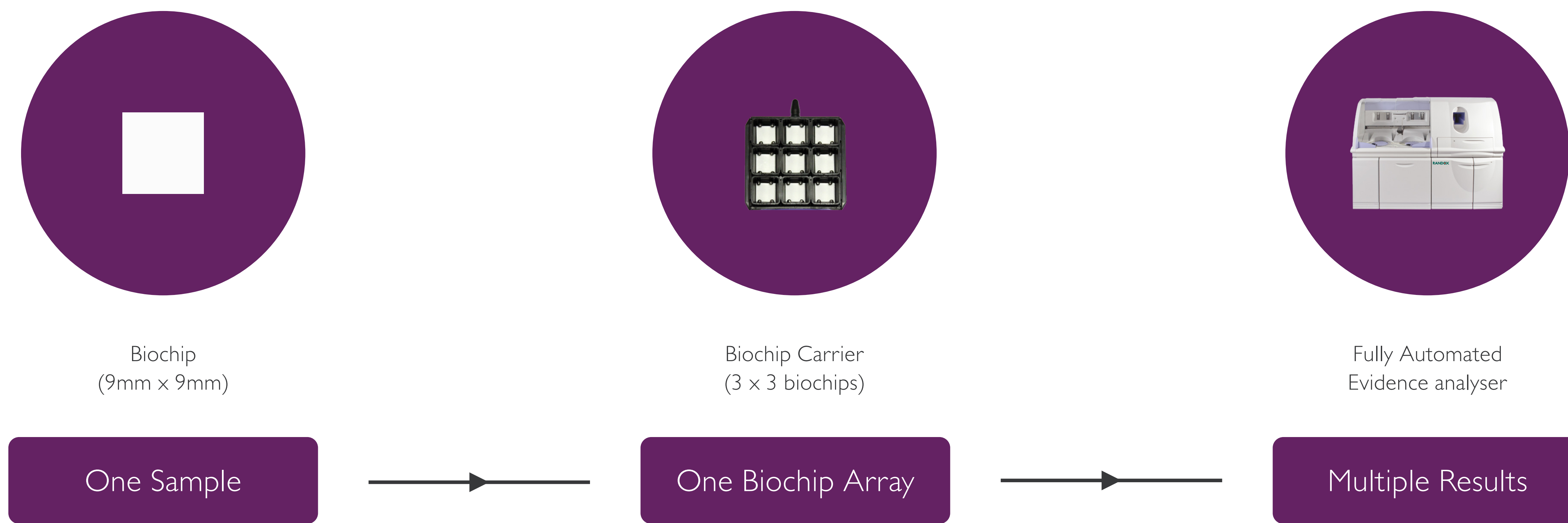
Biochip array technology allows the simultaneous detection of multiple drugs from a single undivided sample, which increases the screening capacity and the result output per sample. Polydrug consumption can be detected and by incorporating new immunoassays on the biochip surface, this technology has the capacity to adapt to the new trends in the drug market.

This study summarises the analytical performance of three different biochip arrays applied to the screening of acetylfentanyl, AH-7921, amphetamine, barbiturates, benzodiazepines (including etizolam and clonazepam), benzoyllecgonine/cocaine, benzylpiperazines, buprenorphine, cannabinoids, carfentanil, dextromethorphan, fentanyl, furanyl fentanyl, meprobamate, mescaline, methamphetamine, methadone, mitragynine, MT-45, naloxone, ocfentanyl, opioids, opiates, oxycodone, phenacyclidine, phenylpiperazines, salvinorin, sufentanil, synthetic cannabinoids (JWH-018, UR-144, AB-PINACA, AB-CHMINACA), synthetic cathinones [mephedrone, methcathinone, alpha-pyrrolidinopentiophenone (alpha-PVP)], tramadol, tricyclic antidepressants, U-47700, W-19, zolpidem.

Methodology

Three different biochip arrays were used (DOA ULTRA, NPSI, NPSII). Simultaneous competitive chemiluminescent immunoassays on a biochip surface applied to the Evidence analyser were employed for the

screening of human urine samples (EV4101, EV4264, EV4269, EV3600, Randox Toxicology Ltd, Crumlin, UK). The results obtained are semi-quantitative.



Results

Drugs of Abuse ULTRA Array (Urine)

Cut-off, Limit of detection (LOD), Typical assay range, Cross-reactivity (CR)

Assay	Cut-off (ng/mL)	LOD (ng/mL)	Compound	Typical assay range* (ng/mL)	CR (>20%)
Amphetamine	200	31.7	S(+)-amphetamine	0-1500	(±)-MDA, PMA HCl, BDB, (±)-Amphetamine, Phentermine
Barbiturates	200	25.1	Phenobarbital	0-900	Secobarbital, Butobarbital, Pentobarbital, Alphenal, Cyclopentobarbital, p-OH-phenobarbital, Butabital, Amobarbital, Barbitol
Benzodiazepines I	100	1.0	Oxazepam	0-400	Temazepam, Nordiazepam, alpha-OH-Alprazolam, Alprazolam, Diazepam, Etizolam, Clonazepam, Nitrazepam, 2-OH-Ethylflurazepam, Prazepam, Midazolam, Flunitrazepam, Flurazepam, Phenazepam, Desalkylflunitrazepam, Lormetazepam, Chlordiazepoxide, Triazolam, Etizolam, N-Desmethylflunitrazepam, Bromazepam
Benzodiazepines 2	100	2.8	Lorazepam	0-500	Phenazepam, Clonazepam, Lorazepam glucuronide
Benzoyllecgonine/Cocaine	150	8.6	Benzoyllecgonine	0-750	Cocaine, m-Hydroxybenzoyllecgonine, Cocacethylene
Buprenorphine Metabolite	5	0.1	Norbuprenorphine	0-25	
Cannabinoids (THC)	20	1.2	(-)-11-nor-9-carboxy-Δ9-THC	0-400	
Dextromethorphan	20	0.6	Dextromethorphan	0-100	Dextromethorphan tartrate salt, (±)-Nordextromethorphan
Fentanyl	2	0.2	Fentanyl	0-40	α-Methylfentanyl, p-Fluorofentanyl, Benzylfentanyl, Butyrylfentanyl HCl, Norfentanyl
Generic Opioids	100	10.4	Oxycodone	0-750	Morphine, Hydrocodone, Ethyl morphine HCl, Codeine, 6-Acetyl-codeine, Dihydrocodeine, Hydromorphone, Desomorphine, Morphine-3β-D-glucuronide, Heroin, 6-MAM
Meprobamate	500	9.6	Meprobamate	0-2500	Carisoprodol
Methadone	300	4.7	Methadone	0-1500	
Methamphetamine	200	7.9	S(+)-methamphetamine	0-1000	PMMA HCl, MDMA, (±)-Methamphetamine
Opiates	200	13.4	Morphine	0-1000	Hydrocodone, Ethyl morphine HCl, Codeine, 6-Acetyl-codeine, Hydromorphone, Desomorphine, Morphine-6β-D-glucuronide, Heroin, 6-MAM
Oxycodone 1	100	3.6	Oxycodone	0-400	Hydrocodone, Noroxycodone
Oxycodone 2	100	0.8	Oxycodone	0-400	Oxymorphone
Phencyclidine	25	0.9	Phencyclidine	0-100	
Tramadol	5	0.9	Tramadol	0-50	O-Desmethyltramadol
Tricyclic Antidepressants (TCA)	100	4.6	Nortriptyline	0-1000	Imipramin N oxide, Imipramine, Trimipramine, Desipramine, Cyclobenzapine, Amitriptyline, Opipramol, Promazine, Maprotiline, Doxepin, Clomipramine, Protriptyline, Cyproheptadine, Lofepamine, Dothiepin, Chlorpromazine
Zolpidem	10	0.5	Zolpidem	0-80	Metabolite I (4-carboxyzolpidem)

*Individual ranges of calibrator batches may vary slightly

Inter-assay precision and recovery

-50% Cut-off
The biochip array recovery ranged from 74% to 129% and the inter-assay precision CV from 4.8% to 17.1%.

Cut-off
The biochip array recovery ranged from 75% to 127% and the inter-assay precision CV from 4.8% to 18.2%.

+50% Cut-off
The biochip array recovery ranged from 75% to 124% and the inter-assay precision CV from 6.9% to 18.2%

EV4101 141217 dm

New Psychoactive Substances I Array (Urine)

Cut-off, Limit of detection (LOD), Typical assay range, Cross-reactivity (CR)

Assay	Cut-off (ng/mL)	LOD (ng/mL)	Compound	Typical assay range* (ng/mL)	CR (>20%)
AB-CHMINACA	2	0.42	AB-CHMINACA	0-30	MA-CHMINACA, MDMB-CHMINACA
AB-PINACA	5	0.08	AB-PINACA N-pentanoic acid	0-76.25	AB-PINACA N-(5-hydroxypentyl) metabolite, AB-PINACA pentanoic acid metabolite, 5-Fluoro-AB-PINACA, AB-CHMINACA metabolite M1A, AB-PINACA N-(4-hydroxypentyl) metabolite, AB-PINACA, ADB-PINACA pentanoic acid metabolite, ADB-PINACA N-(5-hydroxypentyl) metabolite, 5-Fluoro-ADB-PINACA, 5-fluoro-AB-PINACA N-(4-hydroxypentyl) metabolite
α-PVP	1	0.36	α-PVP	0-10	Pyrovalerone, 3,4-Methylenedioxypropylvalerone (MDPV), Naphyrone, α-Pyrrolidinopenthiophenone, 4-Methyl-α-Pyrrolidinobutisophenone (MPBP), 4-Methyl-α-Pyrrolidinohexanophenone (4-MHPHP)
Benzylpiperazines	10	2.41	1-Benzylpiperazine	0-100	1-[4-(Trifluoromethyl)benzyl]piperazine, 4-Hydroxy-benzylpiperazine (p-OH-BZP), 3-(Piperazin-1-ylmethyl)phenol diHCl, 1-Piperonylpiperazine, N-(3-Methylbenzyl)piperazine diHCl
JWH-018	10	0.43	JWH-018	0-300	AM1220, JWH-018 N-(5-hydroxypentyl) metabolite, AM2201, 1-(4-Carboxybutyl)-1H-indol-3-yl(naphthalen-1-yl)methanone (N-carboxybutyl) JWH-018, JWH 200 6-hydroxyindole metabolite, (5'-Carboxy) JWH-018, JWH-018, JWH-073 N-Butanol, JWH 073 N-(4-hydroxybutyl) metabolite, JWH 019 N-(6-hydroxyheptyl) metabolite, JWH-073, (±) JWH 018 N-(4-hydroxypentyl) metabolite, AM2201 N-(4-fluoropentyl) isomer, JWH-200, (±) JWH 073 N-(3-hydroxybutyl) metabolite, JWH 018 N-(3-methylbutyl) isomer, JWH 073 6-hydroxyindole metabolite, JWH-019, JWH 018 6-methoxyindole analog, JWH-022, AM2201 N-(4-hydroxypentyl) metabolite, JWH 018 5-hydroxyindole metabolite, JWH 018 N-(3-hydroxypentyl) β-D-glucuronide, JWH 018 6-hydroxyindole metabolite, JWH 018 N-pentanoic acid metabolite, JWH 073 5-hydroxyindole metabolite, JWH 018 N-(2,2-dimethylpropyl) isomer, AM2201 6-hydroxyindole metabolite, JWH 073 N-(2-methylpropyl) isomer, JWH 073 7-hydroxyindole metabolite, JWH 018 7-hydroxyindole metabolite, JWH 018 N-(2-methylbutyl) isomer, JWH-073 4-butanolic acid metabolite, JWH 019 5-hydroxyindole metabolite, JWH 018 N-(1-methylbutyl) isomer, JWH 398 N-(5-hydroxypentyl) metabolite
Mephedrone	5	0.19	Mephedrone HCl	0-50	Methylone HCl, Methedrone HCl, Fiegledrone HCl, Methcathinone HCl, R(+)-Methcathinone HCl, 3-Fluoromethcathinone HCl
Mescaline	7.5	1.71	Mescaline HCl	0-250	(+/-)-3,4,5-Trimethoxyamphetamine hydrochloride (TMA)
Phenylpiperazines I	7.5	0.17	1-(3-Chlorophenyl)piperazine monohydrochloride (mCPP)	0-75	1-(3-Methylphenyl)piperazine, 1-(2-Chlorophenyl)piperazine HCl, 1-(3-Hydroxyphenyl)piperazine, 1-Phenylpiperazine, 1-(4-Methoxyphenyl)piperazine diHCl, 1-(4-Chlorophenyl)piperazine, Para-fluorophenyl piperazine DiHCl, 1-(4-Methylphenyl)piperazine, 1-(4-Hydroxyphenyl)piperazine
Phenylpiperazines II	7.5	0.29	1-(3-Chlorophenyl)piperazine monohydrochloride (mCPP)	0-75	1-(3-Methylphenyl)piperazine, 1-(2-Chlorophenyl)piperazine HCl, 1-(3-Hydroxyphenyl)piperazine, 1-Phenylpiperazine, 1-(4-Chlorophenyl)piperazine, Para-Fluorophenyl piperazine DiHCl, 1-(4-Methylphenyl)piperazine, 1-(3-Trifluoromethylphenyl)piperazine HCl, 1-(2-Methoxyphenyl)piperazine DiHCl
Salvinorin	0.5	0.02	Salvinorin A	0-10	Salvinorin B
UR-144	5	0.05	UR-144 N pentanoic acid	0-30	A796260, AB-005, A-834735, UR144 N-(5-hydroxypentyl) β-D-glucuronide, UR144 N-(5-hydroxypentyl) metabolite, UR144 N-(4-hydroxypentyl) metabolite, UR144 desalkyl

*Individual ranges of calibrator batches may vary slightly

Inter-assay precision and recovery

-50% Cut-off
The biochip array recovery ranged from 77% to 138% and the inter-assay precision CV from 7.0% to 16.6%.

Cut-off
The biochip array recovery ranged from 86% to 127% and the inter-assay precision CV from 6.1% to 5.8%.

+50% Cut-off
The biochip array recovery ranged from 74% to 112% and the inter-assay precision CV from 5.3% to 10.9%

EV4264 270417 ml

New Psychoactive Substances II array (Urine)

Cut-off, Limit of detection (LOD), Typical assay range, Cross-reactivity (CR)

Assay	Cut-off (ng/mL)	LOD (ng/mL)	Compound	Typical assay range* (ng/mL)	CR (>20%)
AH-7921	1	0.02	AH-7921	0-5	Nor-AH-7921
Buprenorphine metabolite	0.5	0.02	Norbuprenorphine	0-5	
Designer Benzodiazepine assays Clonazepam	2	0.05	Clonazepam	0-15	N-Desmethylflunitrazepam, Delorazepam, 7-Aminoclonazepam, Nitrazepam, Phenazepam
Designer Benzodiazepine assays Etizolam	2	0.05	Etizolam	0-10	Brotizolam, Alpha-OH Etizolam, Estazolam, Deschloroetizolam,
Designer Fentanyl Assays Acetylfentanyl	1	0.04	Ocfentanyl	0-20	Furanyl fentanyl, Acetylfentanyl, Thiofentanyl, Methoxyacetyl Fentanyl, Fentanyl, Butyrylfentanyl, Alpha-Methylfentanyl, Furanylthiofentanyl, Parafluorofentanyl, Tetrahydrofuran Fentanyl, Ortho-Fluorofentanyl, Cyclopentylfentanyl, Para-Fluoroisobutyrylfentanyl, Acrylylfentanyl, Isobutyrylfentanyl, Valeryl fentanyl, 4-Fluoroisobutyryl fentanyl, (±)-cis-3-methylfentanyl, Cis-Mefentanyl, D-Hydroxyfentanyl, (±)-trans-3-methylfentanyl, Para-methoxy-Butyryl fentanyl (HCl),
Designer Fentanyl Assays Carfentanil	0.25	0.07	Carfentanil	0-2.19	Remifenantil Acid, Alfentanil, Norcarfentanil
Designer Fentanyl Assays Furanyl fentanyl	1	0.10	Ocfentanyl	0-20	Furanyl fentanyl, Thiofentanyl, Methoxyacetyl Fentanyl, Fentanyl, Butyrylfentanyl, Alpha-Methylfentanyl, Furanylthiofentanyl, Parafluorofentanyl, Tetrahydrofuran Fentanyl, Ortho-Fluorofentanyl, Cyclopentylfentanyl, Para-Fluoroisobutyrylfentanyl, Benzylfentanyl, Norfentanyl, Acrylylfentanyl, Thienylfentanyl, Isobutyrylfentanyl, Meta-Hydroxy-Acrylylfentanyl, Norfurylfentanyl, Valeryl fentanyl
Designer Fentanyl Assays Ocfentanyl	2	0.13	Ocfentanyl	0-20	Methoxyacetyl Fentanyl, Butyrylfentanyl
Designer Fentanyl Assays Sufentanil	1	0.03	Sufentanil	0-5	Alfentanil, Norsufentanil
Mitragynine	1	0.18	Mitragynine	0-10	
MT-45	2	0.20	MT-45	0-15	3-Hydroxy MT-45
Naloxone	1	0.08	Naloxone	0-20	Naloxone 3-Beta-D Glucuronide
U-47700	10	1.47	U-47700	0-80	N-Desmethyl U-47700
W-19	2	0.31	W-19	0-40	Deschloro W-19

*Individual ranges of calibrator batches may vary slightly

Inter-assay precision and recovery

-50% Cut-off
The biochip array recovery ranged from 88% to 135% and the inter-assay precision CV from 4.4% to 15%.

Cut-off
The biochip array recovery ranged from 79% to 122% and the inter-assay precision CV from 3.6% to 1.6%.

+50% Cut-off
The biochip array recovery ranged from 72% to 109% and the inter-assay precision CV from 3.0% to 12.8%

EV4269/EV4270 040218 pl

Conclusion

The data indicate applicability of biochip array technology to the screening of a broad range of drugs of abuse (including new psychoactive substances) in human urine, which is beneficial during the drug testing process as it reduces the number of samples requiring confirmatory analysis.