

COMPREHENSIVE DRUG SCREENING FROM A SINGLE UNDIVIDED URINE SAMPLE WITH BIOCHIP ARRAYS APPLIED TO EVIDENCE SERIES ANALYSERS

RANDOX TOXICOLOGY

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Introduction

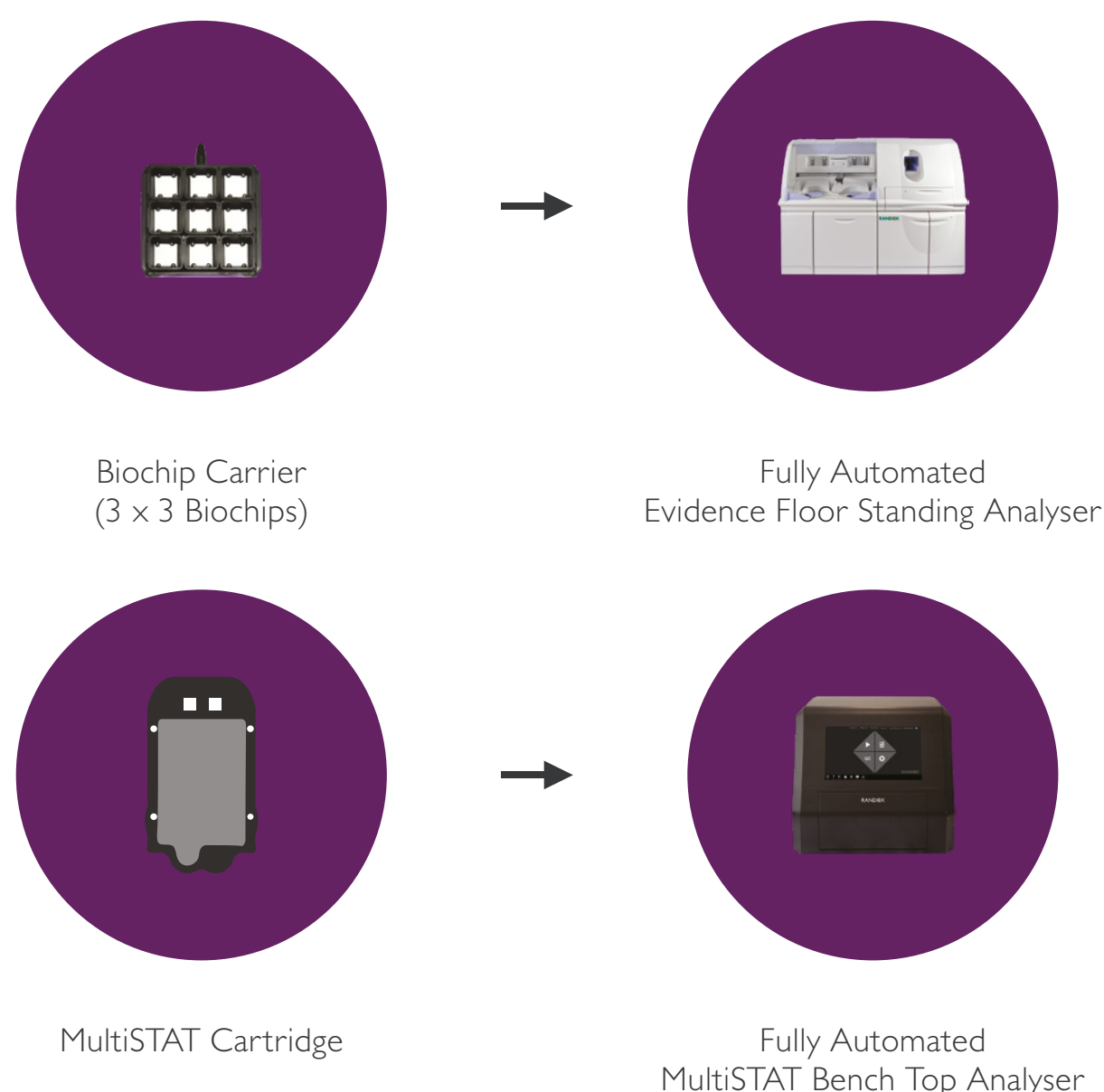
Biochip array technology allows the simultaneous detection of multiple drugs from a single undivided sample, which increases the screening capacity and the result output per sample. Polydrug consumption can be detected and by incorporating new immunoassays on the biochip surface, this technology has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

This study summarises the analytical performance of two biochip arrays, one applicable to the fully automated high throughput floor standing Evidence analyser for the semi-quantitative determination of amphetamine, barbiturates, benzodiazepines, benzoyllecgonine/cocaine, buprenorphine metabolite, cannabinoids, dextromethorphan, fentanyl, meprobamate, methamphetamine, methadone, opiates, oxycodone, phencyclidine, tramadol, tricyclic antidepressants and zolpidem. The other biochip array is applicable to the fully automated benchtop analyser Evidence MultiSTAT for the rapid qualitative detection (under 20 minutes) of alpha-pyrrolidinopentiphenone (alpha-PVP), amphetamine, barbiturates, benzodiazepines, benzoyllecgonine/cocaine, buprenorphine, cannabinoids, ethyl glucuronide (ETG), fentanyl, 6-monoacetylmorphine (6-MAM), methamphetamine, methadone, opiates, oxycodone, synthetic cannabinoids (AB-PINACA, JWH-018, UR-144/XLR-11), tramadol and tricyclic antidepressants from a single urine sample.

Methodology

DOA ULTRA array and DOA MultiSTAT urine array were used (EV4101, EV4193, Randox Toxicology Ltd., Crumlin, UK). Simultaneous competitive chemiluminescent immunoassays on a biochip surface applied to the Evidence or Evidence multiSTAT analysers (EV3600, EV4115, Randox Toxicology Ltd., Crumlin, UK) were employed for the screening of human urine samples.

The results obtained with DOA ULTRA array are semi-quantitative as some immunoassays detect multiple related drugs, which was assessed by determination of the specificity (e.g. barbiturates, benzodiazepines). For DOA MultiSTAT array, sampling against a cut-off sample provides a qualitative result.



Results

Drugs of abuse ULTRA Array (urine) on Evidence

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

Assay	Cut-off (ng/mL)	LOD (ng/mL)	Compound	Typical assay range* (ng/mL)
Amphetamine	200	31.7	S(+)-amphetamine	0-1500
Barbiturates	200	25.1	Phenobarbital	0-900
Benzodiazepines I	100	1.0	Oxazepam	0-400
Benzodiazepines 2	100	2.8	Lorazepam	0-500
Benzoyllecgonine/Cocaine	150	8.6	Benzoyllecgonine	0-750
Buprenorphine Metabolite	5	0.1	Norbuprenorphine	0-25
Cannabinoids (THC)	20	1.2	(-)-11-nor-9-carboxy- Δ^9 -THC	0-400
Dextromethorphan	20	0.6	Dextromethorphan	0-100
Fentanyl	2	0.2	Fentanyl	0-40
Generic Opioids	100	10.4	Oxycodone	0-750
Meprobamate	500	9.6	Meprobamate	0-2500
Methadone	300	4.7	Methadone	0-1500
Methamphetamine	200	7.9	S(+)-methamphetamine	0-1000
Opiates	200	13.4	Morphine	0-1000
Oxycodone 1	100	3.6	Oxycodone	0-400
Oxycodone 2	100	0.8	Oxycodone	0-400
Phencyclidine	25	0.9	Phencyclidine	0-100
Tramadol	5	0.9	Tramadol	0-50
Tricyclic Antidepressants (TCA)	100	4.6	Nortriptyline	0-1000
Zolpidem	10	0.5	Zolpidem	0-80

*Individual ranges of calibrator batches may vary slightly

Inter-assay precision and recovery

-50% cut-off: recovery range 74-129%, inter-assay precision range: 4.8-17.1%; **cut-off:** recovery range: 75-127%, inter-assay precision range: 4.8-18.2%; **+50% cut-off:** recovery range 75-124%, inter-assay precision range: 6.9-18.2%

EV4101_141217_bfm

Cross-reactivity (CR)

Assay	CR (>20%) Compounds
Amphetamine	(\pm)-MDA, PMA HCl, BDB, (\pm)-Amphetamine, Phentermine
Barbiturates	Secobarbital, Butobarbital, Pentobarbital, Alphenal, Cyclopentobarbital, p-OH-phenobarbital, Butalbital, Amobarbital, Barbitol
Benzodiazepines I	Temazepam, Nordiazepam, alpha-OH-Alprazolam, Alprazolam, Diazepam, Estazolam, Clobazam, Nitrazepam, 2-OH-Ethylflurazepam, Prazepam, Midazolam, Flunitrazepam, Flurazepam, Phenazepam, Desalkylflunitrazepam, Lormetazepam, Chlordiazepoxide, Triazolam, Etizolam, N-Desmethylflunitrazepam, Bromazepam
Benzodiazepines 2	Phenazepam, Clonazepam, Lorazepam glucuronide
Benzoyllecgonine/Cocaine	Cocaine, m-Hydroxybenzoyllecgonine, Cocaethylene
Buprenorphine Metabolite	
Cannabinoids (THC)	
Dextromethorphan	Dextromethorphan tartrate salt, (\pm)-Nordextromethorphan
Fentanyl	α -Methylfentanyl, p-Fluorofentanyl, Benzylfentanyl, Butyrylfentanyl HCl, Norfentanyl
Generic Opioids	Morphine, Hydrocodone, Ethyl morphine HCl, Codeine, 6-Acetyl-codeine, Dihydrocodeine, Hydromorphone, Desomorphine, Morphine-3BD-glucuronide, Heroin, 6-MAM
Meprobamate	Carisoprodol
Methadone	
Methamphetamine	PMMA HCl, MDMA, (\pm)-Methamphetamine
Opiates	Hydrocodone, Ethyl morphine HCl, Codeine, 6-Acetyl-codeine, Hydromorphone, Desomorphine, Morphine-6BD-glucuronide, Heroin, 6-MAM
Oxycodone 1	Hydrocodone, Noroxycodone
Oxycodone 2	Oxymorphone
Phencyclidine	
Tramadol	O-Desmethyltramadol
Tricyclic Antidepressants (TCA)	Imipramin N oxide, Imipramine, Trimipramine, Desipramine, Cyclobenzapine, Amitriptyline, Opipramol, Promazine, Maprotiline, Doxepin, Clomipramine, Protryptiline, Cyproheptadine, Lofepamine, Dothiepin, Chlorpromazine
Zolpidem	Metabolite I (4-carboxyzolpidem)

Drugs of Abuse MultiSTAT (urine)

Test Menu and Cut-Offs

Analyte	Cut-off	Analyte	Cut-off
AB-PINACA	2.5 ng/mL	JWH-018	20 ng/mL
α -PVP	5 ng/mL	6-MAM	10 ng/mL
Amphetamine	200 ng/mL	Methadone	300 ng/mL
Barbiturates	200 ng/mL	Methamphetamine	200 ng/mL
Benzodiazepines I	150 ng/mL	Opiate	200 ng/mL
Benzodiazepines II	150 ng/mL	Oxycodone	50 ng/mL
Benzoyllecgonine/Cocaine	150 ng/mL	Tramadol	5 ng/mL
Buprenorphine	1 ng/mL	THC	20 ng/mL
ETG	750 ng/mL	Tricyclic Antidepressants (TCA)	150 ng/mL
Fentanyl	2 ng/mL	UR-144	10 ng/mL
Creatinine	20 mg/dL		

Accuracy & Repeatability

- The accuracy for all analytes was determined by assessing spiked samples at varying concentrations (50 spiked positive samples prepared at concentrations greater than the cut-off, 10 negative spiked samples prepared at concentrations lower than the cut-off and 40 blank negative samples). Each sample was assessed against the cut-off material to determine a positive or negative result. The percentage agreement was calculated as the percentage of correct reports out of the total number of samples analysed (n=100).
- The repeatability for all analytes was determined by assessing control material prepared at the cut-off and at $\pm 50\%$ of the cut-off. Each sample was assessed against the cut-off material twice a day for 10 days, resulting in n=20 results for each sample. The percentage agreement was calculated for the number of samples that correctly reported negative and positive.

Assay	Accuracy		Repeatability	
	Agreement (%)		Agreement (%)	
AB-PINACA	100		97.5	
α -PVP	100		100	
Amphetamine	100		100	
Barbiturates	100		100	
Benzodiazepines I	100		100	
Benzodiazepines II	100		100	
Benzoyllecgonine/cocaine	100		100	
Buprenorphine	96		100	
ETG	100		97.5	
Fentanyl	100		100	
JWH-018	100		100	
6-MAM	100		100	
Methadone	100		100	
Methamphetamine	100		100	
Opiate	100		100	
Oxycodone	100		100	
THC	94		100	
Tramadol	100		100	
TCA	100		100	
UR-144	100		100	
Creatinine	100		100	

EV4193_030818_pt

Sample assessment

Screening of 30 authentic samples (including positive and negative samples) presented the following percentage agreement with LC-MS/MS: 100% (oxycodone), 97% (benzodiazepines, methadone and opiate), 93% (amphetamine, buprenorphine, methamphetamine, and THC), 80% (benzoyllecgonine/cocaine). All samples screened positive for the presence of creatinine (>20mg/dL) indicating that no sample dilution occurred.

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Conclusion

The application of biochip arrays to the Evidence and Evidence MultiSTAT analysers allows the high throughput or rapid screening respectively of a broad range of drugs of abuse from a single urine sample. These systems represent useful analytical tools for urine drug testing by reducing the number of samples requiring confirmatory analysis.