RANDOX TOXICOLOGY

MULTI-ANALYTICAL SCREENING OF DRUGS OF ABUSE AND CREATININE FROM A SINGLE URINE SAMPLE IN LESS THAN TWENTY MINUTES WITH THE FULLY AUTOMATED BIOCHIP ANALYSER EVIDENCE MULTISTAT
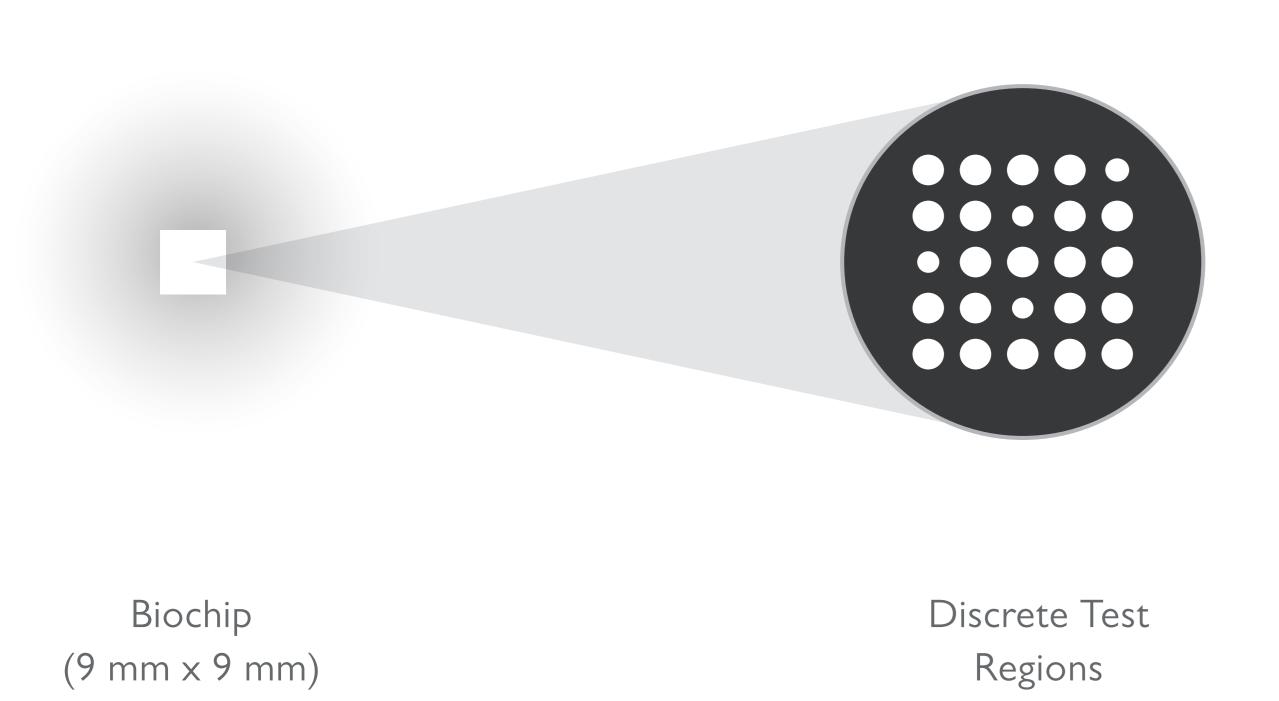
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Introduction

During the drug testing process, a reliable and rapid screening step is important. Furthermore, a multi-analytical approach increases the screening capacity and provides more information from a sample. Urine is the testing sample of choice for many drug tests. Biochip array technology allows the simultaneous detection of multiple drugs of abuse from a single urine sample in less than twenty minutes when applied to the biochip analyser Evidence MultiSTAT. The application reported in this study represents a useful multi-analytical tool for the toxicological analysis.

Methodology

The core of the technology is the biochip (9mm x 9mm) which represents not only the platform on which the capture ligands are immobilized and stabilised, defining microarrays of discrete test sites, but also the vessel in which the simultaneous immunoreactions are performed. Simultaneous chemiluminescent immunoassays were employed and applied to the analyser Evidence MultiSTAT. This fully automated system processes a self-contained cartridge containing all the components required for the immunoassay reactions and has the capacity to assess two biochips in under 20 minutes. Sampling 25µl of urine against a cut-off sample, the results obtained are qualitative.

Biochip (9 mm x 9 mm) — Discrete Test Regions — MultiSTAT Cartridge — Fully Automated MultiSTAT analyser

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
Benzoylecgonine/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		

Results

Accuracy

Accuracy 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).

Repeatability

Repeatability was determined by assessing control material prepared at the cut-off and at ±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		Spike +	Spike -	Agreement (%)
AB-PINACA	+	50	0	100
	-	0	50	
α-PVP	+	50	0	100
	-	0	50	
Amphetamine	+	50	0	100
	-	0	50	
Barbiturates	+	50	0	100
	-	0	50	
Benzodiazepines I	+	50	0	100
	-	0	50	
Benzodiazepines II	+	50	0	100
	-	0	50	
Benzoylecgonine/Cocaine	+	50	0	100
	-	0	50	
Buprenorphine	+	46	0	96
	-	4	50	
ETG	+	50	0	100
	-	0	50	
Fentanyl	+	50	0	100
	-	0	50	
JWH-018	+	50	0	100
	-	0	50	
6-MAM	+	50	0	100
	-	0	50	
Methadone	+	50	0	100
	-	0	50	
Methamphetamine	+	50	0	100
	-	0	50	
Opiate	+	50	0	100
	-	0	50	
Oxycodone	+	50	0	100
	-	0	50	
THC	+	44	0	94
	-	6	50	
Tramadol	+	50	0	100
	-	0	50	
TCA	+	50	0	100
	-	0	50	
UR-144	+	50	0	100
	-	0	50	
Creatinine	+	100	0	100
	-	0	50	

Assay		-50% cut-off	Cut-off	+50% cut-off	Agreement (%)
AB-PINACA	+	0	8	19	97.5
	-	20	12	1	
α-PVP	+	0	10	20	100
	-	20	10	0	
Amphetamine	+	0	7	20	100
	-	20	13	0	
Barbiturates	+	0	8	20	100
	-	20	12	0	
Benzodiazepines I	+	0	12	20	100
	-	20	8	0	
Benzodiazepines II	+	0	11	20	100
	-	20	9	0	
Benzoylecgonine/Cocaine	+	0	12	20	100
	-	20	8	0	
Buprenorphine	+	0	8	20	100
	-	20	12	0	
ETG	+	0	8	19	97.5
	-	20	12	1	
Fentanyl	+	0	13	20	100
	-	20	7	0	
JWH-018	+	0	12	20	100
	-	20	8	0	
6-MAM	+	0	14	20	100
	-	20	6	0	
Methadone	+	0	12	20	100
	-	20	8	0	
Methamphetamine	+	0	12	20	100
	-	20	8	0	
Opiate	+	0	12	20	100
	-	20	8	0	
Oxycodone	+	0	7	20	100
	-	20	13	0	
THC	+	0	14	20	100
	-	20	6	0	
Tramadol	+	0	12	20	100
	-	20	8	0	
TCA	+	0	11	20	100
	-	20	9	0	
UR-144	+	0	9	20	100
	-	20	11	0	
Creatinine	+	0	10	20	100
	-	20	10	0	

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% (benzoylecgonine/cocaine). All samples screened positive for the presence of creatinine (>20mg/dL) indicating that no sample dilution occurred.

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Conclusion

Data indicate optimal analytical performance and applicability of biochip array technology to the rapid (less than 20 minutes) simultaneous detection of multiple drugs of abuse and creatinine from a single urine sample, when applied to the Evidence MultiSTAT. This system represents a reliable and quick multi-analytical screening tool for a wide range of substances, which facilitates the process in toxicological analysis.