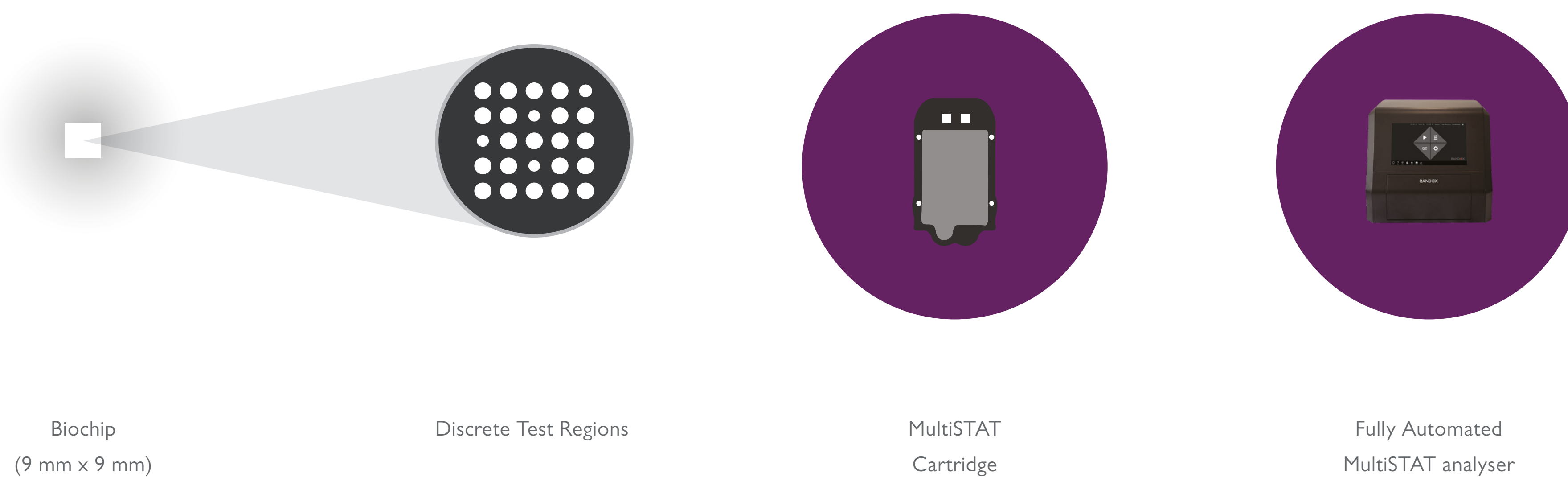


## Introduction

Biochip array technology facilitates the screening step in the drug testing process as it allows multiplex screening testing, therefore reducing the number of samples requiring confirmatory analysis. A biochip array was designed for the simultaneous detection of classical, prescription and synthetic drugs from a single urine sample (creatinine is also included). The application of this biochip array to the fully automated analyser Evidence MultiSTAT, enables the multi-analytical screening within twenty minutes. This study reports analytical performance of this application.

## Methodology

Simultaneous competitive chemiluminescent immunoassays on a biochip surface were applied to the Evidence MultiSTAT analyser (EV4193, EV4115, Randox Toxicology Ltd., Crumlin, UK) This 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 twenty minutes. By sampling against a cut-off sample, the results are qualitative.



Test Menu	
AB-PINACA	JWH-018
α-PVP	6-MAM
Amphetamine	Methadone
Barbiturates	Methamphetamine
Benzodiazepines I	Opiate
Benzodiazepines II	Oxycodone
Benzoyllecgonine/Cocaine	THC
Buprenorphine	Tramadol
ETG	Tricyclic antidepressants (TCAs)
Fentanyl	UR-144
Creatinine	

## Results

### 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

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).

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	
Benzoyllecgonine/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	

### 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	+	-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	
Benzoyllecgonine/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	

EV4193 180618.pl

### 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

Results indicate optimal analytical performance of the reported biochip array applied to the fully automated analyser Evidence MultiSTAT. Classical, prescription and synthetic drugs are simultaneously detected from a single undivided urine sample within twenty minutes, facilitating the testing process.