VALIDATION OF THE RANDOX DRUGS OF ABUSE (DOA) ARRAY FOR URINE

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Introduction

Rates of visits to the Emergency departments due to opioid overdose increased by 28.5% across the U.S. in 2020, compared to 2018 and 2019[1]. The Centers for Disease Control and Prevention (CDC) also recorded 75,673 deaths in the 12-month period ending in April 2021, up from 56,064 the year before[2]. Overdose deaths from synthetic opioids (primarily fentanyl) and psychostimulants such as methamphetamine increased in the 12-month period ending in April 2021.

Cocaine deaths also increased, as did deaths from natural and semi-synthetic opioids. Randox developed a Biochip Array for the simultaneous detection of DoAs i.e., Monoacetylmorphine (6-MAM), Cannabinoids (THC), Amphetamine (AMPH), Barbiturates (BARB), Buprenophine (BUP), Benzyolecgonine/Cocaine (BZG), Fentanyl (FENT), Lorazepam (LORAZ), Methadone (MDONE), Methamphetamine (MAMP), Opiate (OPI), Oxazepam (OXAZ), Oxycodone (OXY), Tramadol (TRAM), and Phencyclidine (PCP) with Creatine as a control.

Methodology

The Randox DoA Array for 15 drugs was developed and validated on the Fully Automated Evidence MultiSTAT which uses a self-contained reagent cartridge. Precision and reproducibility of the 15 drugs of abuse assays were assessed with a cut off characterisation study.

Spiked samples were prepared at ±50%, ±75% and ±100% of the cut off (Cut off; FENT 1ng/ml, MAMP 500ng/ml, BARB 200ng/ml, OXAZ 200ng/ml, LORAZ 200ng/ml, MDONE 300ng/ml, OPI 300ng/ml, BZG 150ng/ml, OXY 100ng/ml, TRAM 200ng/ml, THC 50ng/ml, PCP 25ng/ml, AMPH 500ng/ml, BUP 5ng/ml, and 6-MAM 10ng/ml). Each sample was assessed twice a day against the cut off sample, for 20 days (n=40). % Agreement to expected spike value was determined at each concentration.

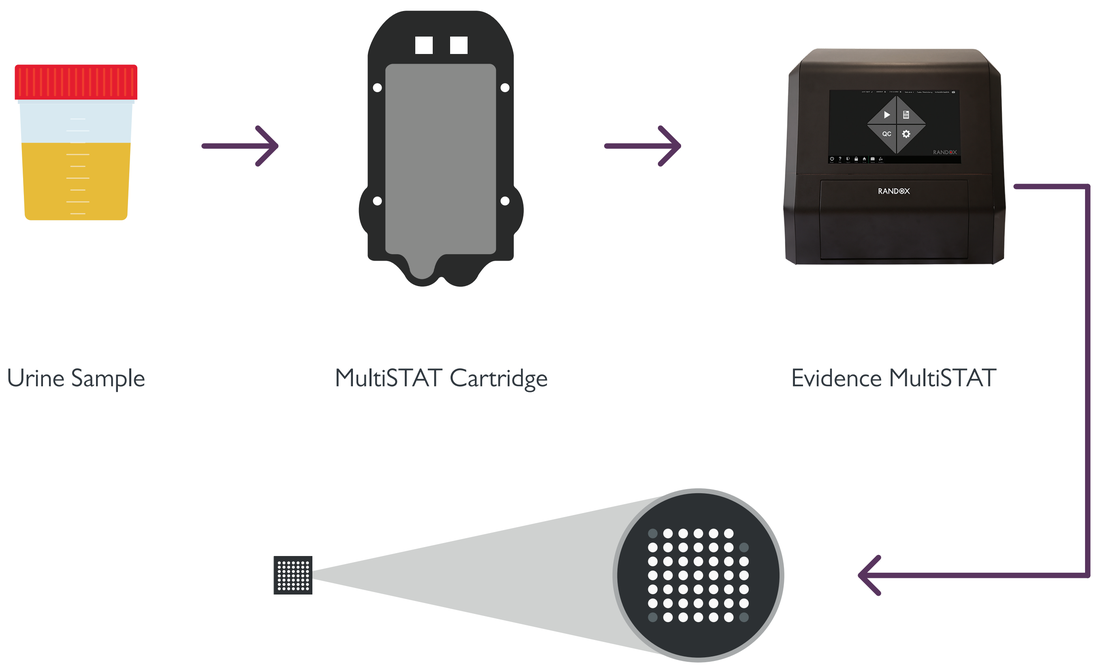

Figure 1: Randox Evidence MultiStat used for the simultaneous detection of 15 DoAs in urine.

In order to evaluate the performance of this DoA Array on the Evidence MultiSTAT, a total of 611 blind urine samples collected by Kaiser Permanente Laboratory previously analyzed on LC-MS/MS were tested on the MultiSTAT DOA Urine 16-Plex Array. % Agreement to LC-MS/MS was determined for all 15 drugs of abuse assays. The number of blind urine samples varied for each analyte as follows; 6-MAM **(244)**, THC **(97)**, AMPH **(127)**, BARB **(96)**, BUP **(292)**, BZG **(128)**, FENT **(291)**, LORAZ **(151)**, MDONE **(291)**, MAMP **(137)**, OPI **(292)**, OXAZ **(151)**, OXY **(292)**, TRAM **(291)**, THC **(97)**, and PCP **(136)**.

References

1.W.E. Soares et al. Annals of Emergency Medicine, 2021; DOI: 10.1016/j.annemergmed.2021.03.013. Emergency Department Visits for Nonfatal Opioid Overdose During the COVID-19 Pandemic Across Six US Health Care Systems.

2.https://www.cdc.gov/nchs/pressroom/nchs_press_releases/2021/20211117.htm

3.Milone MC. J Med Toxicol. 2012 Dec;8(4):408-16.

Results

Below **(Table 1)** are the assay responses of the Randox DoA Array for the simultaneous detection of 15 drugs. All assays had a 100% conformity to expected results except for 10% of 0.5 ng/ml Fentanyl (just below the Cut-Off) run. Therefore, out of the 3,600 test runs there was a 99.88% conformity of results.

% Cut Off	No. of Runs	AMPH	BARB	BUP	6-MAM	BZG	FENT	LORAZ	MDONE
-100%	40	100	100	100	100	100	100	100	100
-75%	40	100	100	100	100	100	100	100	100
-50%	40	100	100	100	100	100	90	100	100
+50%	40	100	100	100	100	100	100	100	100
+75%	40	100	100	100	100	100	100	100	100
+100%	40	100	100	100	100	100	100	100	100

% Cut Off	No. of Runs	MAMP	OXAZ	OXY	TRAM	THC	PCP	OPI
-100%	40	100	100	100	100	100	100	100
-75%	40	100	100	100	100	100	100	100
-50%	40	100	100	100	100	100	100	100
+50%	40	100	100	100	100	100	100	100
+75%	40	100	100	100	100	100	100	100
+100%	40	100	100	100	100	100	100	100

Table 1. Assay performance of the Randox DoA Biochip Array ±50%, ±75%, ±100% of Cut Off.

The MultiSTAT DoA Array was evaluated at Kaiser Laboratory and confirmed the results with LC-MS/MS. The results showed a very high agreement between the two methods ranging from 90% to 99% with an average of 96% ±2.9. Only Fentanyl and the Opiates assays had the lowest at 90%, though still a very high correlation as shown in **Figure 2.**

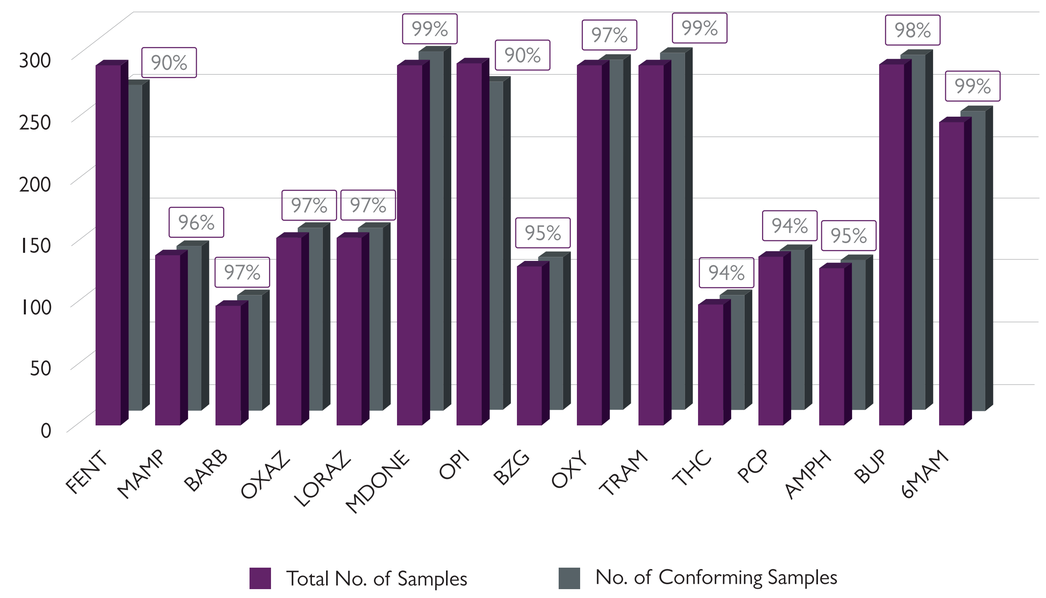

Figure 2. Illustration of the % agreement of DoA Array results confirmed by LC-MS/MS as indicated by the number of conforming results.

Conclusion

The Randox DOA Urine 16-Plex Biochip Array on the Evidence MultiSTAT analyser demonstrated a high level of precision and reproducibility, together with excellent agreement with current urine LC-MS/MS confirmation methods used in clinical practice. Compared to other pre-configured screening panels, the array has added testing for fentanyl and tramadol, two widely prescribed opioid drugs making it an attractive option for laboratories to meet the needs of their communities during the current opioid crisis[3].