K55 Comparison of the Randox® Evidence Drugs of Abuse Custom Array VIII Biochip With Accurate Mass Screening III: Meprobamate (MPB), Methadone (MTD), Tramadol (TRM), and Zolpidem (ZPD)

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After attending this presentation, attendees will better understand the comparison of the results obtained between the custom biochip assays for MPB, MTD, TRM, and ZPD with Liquid Chromatography/Time-Of-Flight (LC/TOF) accurate mass screening and Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) or Gas Chromatography/Mass Spectrometry (GC/MS) confirmation.

This presentation will impact the forensic science community by allowing attendees to assess the usefulness of the several assays incorporated in the Randox® Evidence Drugs of Abuse Custom Array VIII Biochip for drug screening in Driving Under the Influence of Drugs (DUID) cases.

Introduction: Blood specimens collected in suspected DUID cases in the State of Michigan are routinely screened for drugs by the Michigan State Police using a Randox® Evidence Analyser and a Drugs of Abuse Custom Array VIII Biochip employing chemiluminescent immunoassay technology. The custom chip is embedded with 14 different antibodies to desired target analytes in discrete testing regions. As part of a workload reduction project, specimens that screened positive for one or more analytes on the biochip were sent to NMS Labs for analysis by LC accurate mass screening and confirmation of presumptive positive findings.

Methods: Blood specimens were analyzed with cutoff concentrations as noted in Table 1 using the Randox® Biochip as the initial screen, LC/TOF accurate mass screening as a rescreen, and confirmations by either LC/MS/MS or GC/MS. Only cases which tested positive above the LC/TOF decision point were confirmed.

Table 1: Biochip cut-off concentrations, LC/TOF Decision Points and LC/MS/MS, and GC/MS Reporting Limits (ng/mL)

	Target Analytes (ng/mL)						
Method	**Meprobamate**	**Carisoprodol**	**Methadone**	**EDDP**	**Tramadol**	**O-DMT**	**Zolpidem**
Biochip	25	*	10	*	5	*	5
LC/TOF	1000	200	50	50	20	25	10
LC/MS/MS	-	-	-	-	20	20	4
GC/MS	1000	200	50	50	-	-	-

*The manufacturer reported cross-reactivities of 88% for carisoprodol (MPB assay), <0.01% for EDDP and EDMP (MTD assay), 32.8%, 11.9%, 2.7% for the (+/-)N,O didesmethyl, O-desmethyl, (+/-)N-desmethyl tramadol metabolites (TRM assay), and 31% for phenyl-4carboxy zolpidem (ZPD assay).

Results: A total of 1,858 blood specimens were tested. Table 2 summarizes the data obtained by the biochip assays and the LC/TOF screen. Although the LC/TOF decision point for the MPB assay had a much higher decision point than the Biochip cutoff, false negatives were minimized due to the cross-reactivity of carisoprodol, which was present in 94% of the positive cases. For all analytes, most cases for which the Biochip was positive and the LC/TOF was "negative" had an LC/TOF response for the drug, but below the decision point; however, since LC/TOF was considered the screening test for testing purposes at NMS Labs, cases with analytes below the LC/TOF decision point were not confirmed and were not considered as true positives in the calculations. For reference, the values in Table 2 in parenthesis are "true" false positive cases (positive Biochip, no LC/TOF response). All positive results by LC/TOF were confirmed by LC/MS/MS or GC/MS.

Table 2: Results: Biochip and LC/TOF screen (parenthetical data are cases for which no LC/TOF response was observed).

MPB			MTD			TRM			ZPD		
	TOF +	TOF –		TOF +	TOF -		TOF +	TOF -		TOF +	TOF -
Chip +	257	20 (7)	Chip +	146	17 (2)	Chip +	136	29 (3)	Chip +	104	12 (0)
Chip –	0	1581	Chip –	0	1695	Chip –	0	1693	Chip –	0	1742

Conclusions: The percent agreement between the MBP, MTD, TRM, and ZPD Randox® Drugs of Abuse Custom Array VIII Biochip and an LC/TOF screen were 98.9%, 99.0%, 98.4%, and 99.3%, respectively. The specificity and sensitivity for the assays were as follows: MBP (100%, 92.8%), MTD (100%, 89.6%), TRM (100%, 82.4%), and ZPD (100%, 89.6%). Based on the LC/TOF responses, had the LC/TOF decision points been more aligned with the Biochip cutoff concentrations, sensitivity would have increased although there may have been some loss of specificity.

Randox® Evidence, Accurate Mass Screeening, Drug-Impaired Driving

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