K9 Validation of the Neogen® Enzyme-Linked Immuno-Sorbent Assay (ELISA) Cocaine/Benzoylecgonine-2 Kit for Whole Blood and Urine Specimens

Nicholas B. Tiscione, MS, West Palm Beach, FL 33406*

Learning Overview: After attending this presentation, attendees will understand the performance of the Neogen® ELISA Cocaine/Benzoylecgonine-2 kit for screening whole blood and urine specimens as evaluated by a validation based on the Scientific Working Group for Forensic Toxicology (SWGTOX) Standard Practices for Method Validation in forensic toxicology laboratories.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by demonstrating the validation of an ELISA method based on the SWGTOX standard.

Objective: The validation of a semi-quantitative method for the rapid screening of whole blood and urine specimens by a Dynex DSX® Automated ELISA System using the Neogen® Cocaine/Benzoylecgonine-2 kit.

Method: Whole blood samples were diluted 1:5 with manufacturer-provided buffer before being loaded onto the instrument, then further diluted 1:2 by the instrument. Urine samples were diluted 1:20 with buffer by the instrument. The diluted sample volume added to each well was 15µL and 10µL for whole blood and urine, respectively. For all other parameters (e.g., incubation times, reagent volumes), the manufacturer assay instructions were followed. Performance of the assay was evaluated at decision points of 50ng/mL and 25ng/mL for whole blood and 300ng/mL, 150ng/mL, and 50ng/mL for urine. The validation included the evaluation of sensitivity, precision, specificity, carryover, plate drift, ruggedness/robustness, and a case sample comparison.

Results: Carryover was evaluated by running three replicates of a blank matrix control following a positive matrix control at 10µg/mL for both blood and urine. Carryover was not detected in the assay. The sensitivity was evaluated by replicate analysis of a blank matrix control to determine the theoretical Limit Of Detection (LOD) and by the analysis of standards at successively lower levels to determine an experimental LOD. The theoretical LOD was determined to be 16ng/mL for blood and 23 ng/mL for urine. The experimental LOD was determined to be 25ng/mL for both blood and urine. Precision was evaluated at 0ng/mL, 12.5ng/mL, 25ng/mL, 37.5ng/mL, 50ng/mL, 75ng/mL, and 100ng/mL for blood and 0ng/mL, 25ng/mL, 50ng/mL, 75ng/mL, 150ng/mL, 225ng/mL, 300ng/mL, and 450ng/mL for urine with three replicates at each level over five separate runs (n=15). The Coefficient of Variation (CV) was less than or equal to 6.1% for blood and urine at all studied concentrations. The mean response ±2 Standard Deviations (SD) at each decision point for both blood and urine at 50ng/mL and 300ng/mL, respectively, did not overlap with the mean response ±2 SD of standards prepared at ±50% of the concentration of the decision points. Overlap was observed at decision points of 25ng/mL in blood and 150ng/mL and 50ng/mL in urine. Specificity was evaluated for cocaine and cocaethylene in blood by the analysis of negative matrix samples fortified at 10µg/mL, 100ng/mL, 70ng/mL, and 50ng/mL. In urine, concentrations of 10µg/mL, 600ng/mL, 400ng/mL, and 300ng/mL were employed. Observed cross reactivity was lower than that reported by the manufacturer. Cocaine cross reactivity was estimated to be 44% for whole blood and 40% for urine. Cocaethylene cross reactivity was estimated to be 46% for whole blood and 43% for urine. No interference was observed from screening known authentic samples, which contained 22 commonly identified drugs and metabolites. Two out of five positive blood specimens and one out of five urine specimens were identified as being negative by the assay, with responses just above the cutoff. These specimens had benzoylecgonine concentrations near the cutoff and had been in storage for 1–35 months. Plate drift was evaluated by analyzing 24 replicates at the validated decision point for each matrix. The number of replicates analyzed was greater than the number of samples run in routine casework. Plate drift was not observed.

Whole Blood (n=15)	Level (ng/mL)	Mean O.D.	SD	CV (%)	Mean + 2 SD	Mean – 2 SD
	0	0.898	0.038	4.2	0.974	0.822
	25	0.693	0.031	4.5	0.755	0.631
	50	0.568	0.026	4.6	0.620	0.516
	75	0.460	0.028	6.1	0.516	0.404
	100	0.408	0.023	5.6	0.454	0.362

Urine (n=15)	Level (ng/mL)	Mean O.D.	SD	CV	Mean + 2 SD	Mean – 2 SD
	0	1.166	0.044	3.8	1.254	1.078
	150	0.579	0.030	5.2	0.639	0.519
	300	0.407	0.017	4.2	0.441	0.373
	450	0.310	0.019	6.1	0.348	0.272

Conclusion: The Neogen® Cocaine/Benzoylecgonine-2 ELISA kit is a precise, specific, and rapid screening procedure to detect benzoylecgonine in blood and urine.

Cocaine, Validation, Enzyme-Linked Immuno-Sorbent Assay