K14 Validation of the Neogen® Enzyme-Linked Immuno-Sorbent Assay (ELISA) Fentanyl Ready-to-Use (RTU) Kit for Whole Blood and Urine Specimens

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After attending this presentation, attendees will understand the performance of the Neogen® ELISA Fentanyl RTU kit for screening whole blood and urine specimens as evaluated by the Scientific Working Group for Forensic Toxicology (SWGTOX) Standard Practices for Method Validation in Forensic Toxicology Laboratories guidelines.

This presentation will impact the forensic science community by demonstrating the validation of an ELISA method following SWGTOX guidelines.

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® Fentanyl (RTU) Kit.

Method: Neogen® Fentanyl kit assay instructions for incubation times, reagent volumes, and sample volumes were followed. Whole blood samples were diluted 1:5 with buffer before being loaded onto the instrument. Urine samples were diluted 1:2 with buffer by the instrument. Performance of the assay was evaluated at two decision points for each matrix (low control and cutoff control). Urine was evaluated at 1ng/mL and 5ng/mL and blood was evaluated at 0.5ng/mL and 1ng/mL. SWGTOX guidelines were followed for the validation of the assay. The validation included the evaluation of sensitivity, repeatability, 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 500ng/mL for both blood and urine. Carryover was not detected in the assay. The sensitivity for this method 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 0.18ng/mL for blood and 0.03ng/mL for urine. The experimental LOD was determined to be 0.5ng/mL for both blood and urine assays. Repeatability was evaluated at 0.25ng/mL, 0.5ng/mL, 0.75ng/mL, 1ng/mL, and 1.5ng/mL for blood and 0.5ng/mL, 1ng/mL, 1.5ng/mL, 2.5ng/mL, 5ng/mL, and 7.5ng/mL for urine with three replicates at each level over five separate runs. The mean response ±2 Standard Deviations (SD) at each decision point for both blood and urine did not overlap with the mean response ±2 SD of standards prepared at ±50% of the concentration of the decision points. The repeatability was determined by calculating the Coefficient of Variation (CV) for 15 inter-run replicate measurements of each assay at each concentration. The CV was less than or equal to 3% for blood and 6% for urine. Specificity was evaluated in blood and urine by the analysis of negative matrix samples spiked at 250ng/mL, 500ng/mL, and 1,000ng/mL of norfentanyl and 5ng/mL, 10ng/mL, and 25ng/mL of acetyl fentanyl. Observed cross reactivity was similar to that stated by the manufacturer. For acetyl fentanyl, cross reactivity was between 29% and 35% for whole blood and between 50% and 59% for urine. For norfentanyl, cross reactivity was between 0.04 and 0.17% for whole blood and between 0.05 and 0.20% for urine. There were no false positives for fentanyl resulting from screening known samples, which contained morphine, hydromorphone, buprenorphine, hydrocodone, oxycodone, and oxymorphone. Two urine cases and one blood case containing fentanyl were positively identified. Plate drift was evaluated by analyzing 24 replicates at the concentration of the cutoff control 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
	0.25	1.848	0.029	1.6	1.906	1.790
Low Control	0.5	1.663	0.036	2.2	1.735	1.591
	0.75	1.528	0.026	1.7	1.580	1.476
Cutoff Control	1	1.379	0.029	2.1	1.437	1.321
	1.5	1.196	0.029	2.4	1.254	1.138

Urine (n = 15)	Level (ng/mL)	Mean O.D.	SD	CV	Mean + 2 SD	Mean – 2 SD
	0.5	1.451	0.042	2.9	1.535	1.366
Low Control	1	1.148	0.027	2.3	1.201	1.094
	1.5	0.984	0.020	2.0	1.024	0.943
	2.5	0.719	0.024	3.4	0.768	0.671
Cutoff Control	5	0.488	0.027	5.6	0.542	0.433
	7.5	0.371	0.008	2.1	0.386	0.355

Conclusion: The Neogen® Fentanyl ELISA kit is a highly sensitive, specific, and rapid screening procedure to detect fentanyl in blood and urine.

Fentanyl, Validation, ELISA