ARK™ Fentanyl Assay for the Beckman Coulter™ AU680 Automated Clinical Chemistry Analyzer

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Background: Fentanyl is a highly addictive potent synthetic opioid that is widely used for chronic pain management and surgical anesthesia. The drug is a controlled Schedule II substance and was introduced in 1960 as a replacement for other opioids in cardiac surgery. Currently, the drug is available as injectable solution for surgical anesthesia and transdermal patches at concentrations of 25, 50, 75, 100 mg/h for chronic pain management. Other than medical applications, fentanyl has also been sold to drug users, primarily heroin abusers and resulted in hundreds of overdoses. The severity of the situation became apparent when the above average numbers of overdoses were observed in many regions in United States. Since fentanyl is 50-100 times more potent than heroin and present in biological samples at very low concentrations, administration and monitoring of fentanyl present a great challenge in clinical and forensic laboratories. There is an increasing need for a high throughput screening method for the detection of fentanyl in human urine.

Methods: The ARK™ Fentanyl Assay is a liquid stable, homogeneous enzyme immunoassay, intended for the qualitative and/or semi-quantitative determination of fentanyl in human urine at a cutoff concentration of 1.0 ng/mL on automated clinical chemistry analyzers. Two reagents, calibrators (0.0, 1.0, 2.0, 4.0, and 10.0 ng/mL) and controls (0.5 and 1.5 ng/mL) compose the test system. The 1.0 ng/mL Calibrator is the Cutoff for distinguishing “positive” from “negative” samples. Precision over 20 days, histogram overlap analysis of Control and Cutoff concentrations, recovery and specificity were evaluated on the Beckman Coulter™ AU680.

Results: Semi-quantitative precision was determined for 0.5 (11.3%CV), 1.0 (5.5%CV) and 1.5 (6.0%CV) ng/mL. Qualitative determination of fentanyl in Low and High controls did not overlap with the Cutoff by histogram analysis. Recovery of fentanyl ranged from 91.6% (0.75 ng/mL) to 104.7% (6.0 ng/mL). Norfentanyl metabolite tested positive at 300.0 ng/mL. Fentanyl analogues despropionylfentanyl, hydroxyfentanyl, acetylfentanyl, butyrylfentanyl, carfentanil, and sufentanil tested positive at 75.0, 1.0, 1.0, 2.0, 500.0 and 600.0 ng/mL respectively. Other opiates were not crossreactive. The sensitivity (true positive, 100 samples) and specificity (true negative, 50 samples) was 96.2% and 98.0%, respectively, versus LC-MS/MS (fentanyl cutoff 0.2 ng/mL).

Conclusions: ARK Fentanyl Assay determines fentanyl in human urine accurately and sensitively in either semi-quantitative or qualitative modes with fast turn-around times. Detection of fentanyl use in pain management, compliance or misuse/abuse with a superior cutoff concentration for a screening assay is an important new addition to clinical chemistry.