K26 Screening for Fentanyl and Its Analogues in Postmortem Specimens Using the OraSure Fentanyl Enzyme-Linked Immuno-Sorbent Assay (ELISA) Direct Kit Assay

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After attending this presentation, attendees will better understand the performance characteristics of a commercially available fentanyl immunoassay screening test for the routine screening of postmortem specimens for a range of both structurally related and structurally unrelated compounds.

Fentanyl is a synthetic opioid that was first introduced into medicinal practice in 1963. Unfortunately, use and abuse of a range of synthetic opioids, including fentanyl, has contributed to an increase in fatalities across the United States of America. Within the New York City Office of Chief Medical Examiner, several structurally-related drugs have been identified in addition to illicit fentanyl, including acetyl fentanyl, furanyl fentanyl, and 4-ANPP. U-47700 has also been identified in a number of cases and although it is a synthetic opioid, it is structurally unrelated to fentanyl. The challenge for all forensic toxicology laboratories is to ensure their testing protocols are sufficiently robust to identify new and emerging drugs and adapt their protocols as necessary to meet this requirement.

This presentation will impact the forensic science community by providing cross-reactivity data for new synthetic opioids and the assay efficiency for the detection of different synthetic opioids in postmortem casework.

The OraSure Fentanyl ELISA direct kit assay was evaluated using the Dynex DSX automated microplate analyzer. The target analyte was fentanyl and in-house positive controls were prepared by fortifying drug-free matrices (blood, serum, and urine) at 0.5ng/mL, 1.0ng/mL, and 1.5ng/mL and 0.5 ng/g, 1.0ng/g, and 1.5ng/g for tissues. Each of the synthetic opioids (acetyl fentanyl, butyryl fentanyl, furanyl fentanyl, isobutyryl fentanyl, norfentanil, parafluorobutyryl fentanyl, sulfentanil, valeryl fentanyl, 3-methyl fentanyl, 4-methoxy butyryl fentanyl, U-4770, MT-45, and W-18) were prepared at 0.5ng/mL, 1.0ng/mL, 2.0ng/mL, and 10ng/mL to evaluate cross-reactivity in comparison to the target compound fentanyl. Postmortem specimens were diluted using forensic diluents provided with the kit at 1:5 for blood and serum, 1:3, 1:5, and 1:10 for tissues, and 1:60 for urine. A total of 183 postmortem cases were screened using ELISA and confirmed for the presence or absence of synthetic opioids using a combination of gas chromatography-mass spectrometry (GC/MS), liquid chromatrography-time of flight (LC/TOF) and liquid chromatography-tandem mass spectrometry (LC/MS/MS).

The cross-reactivities for the following drugs were close to or greater than 100%: acetyl fentanyl, butyryl fentanyl, furanyl fentanyl, parafluorobutyryl fentanyl, 4-methoxybutyryl fentanyl, valeryl fentanyl, isobutyryl fentanyl, and 3-methyl fentanyl. Norfentanyl, sufentanil, W-18, U-47700, and MT-45 did not cross-react. Of the 183 cases screened by ELISA and confirmed by mass spectrometry, there were three false negative and five false positive with an overall efficiency of 95%.

Synthetic Opioids, ELISA, Cross-Reactivity