K21 Evaluation of the Immunalysis® Fentanyl ELISA Assay for Use in Screening Postmortem Blood

Denice M. Teem, BS, Daniel S. Isenschmid, PhD, Bradford R. Hepler, PhD, and Carl J. Schmidt, MD, Wayne County Medical*
Examiner's Office, 1300 East Warren Avenue, Detroit, MI 48207

After attending this presentation, attendees will be aware of a rapid ELISA screening test for fentanyl in postmortem blood that is sensitive, specific, and efficient.

This presentation will impact the forensic community and/or humanity by providing the toxicologist with data to aid in the selection of a reliable screening assay for fentanyl in postmortem blood.

Since 1999, the Wayne County Medical Examiner's Office (WCMEO) has routinely looked for fentanyl in its GC/MS screen (LOD, 5 ng/mL). The incidence of cases in which fentanyl was identified was 6 (1999), 3 (2000), 7 (2001), 12 (2002), 20 (2003) and 29 (2004). These gradual increases were largely due to increased use and abuse of fentanyl patches and lollipops. Fentanyl confirmation and quantitation had been performed by a reference laboratory until May 2005 when the WCMEO developed a GC/MS-SIM confirmation method (LOD, 1ng/mL). Due to an outbreak of fentanyl-laced cocaine and heroin in the last 3 months of 2005, the incidence of fentanyl increased to 63 in 2005 and 132 for the first six months of 2006. Due to the surge in fentanyl-related cases, it was necessary to add an immunoassay to allow the laboratory to rapidly identify potential fentanyl-related cases and to expand its fentanyl screening protocol to all autopsied cases.

The Immunalysis® ELISA fentanyl assay was evaluated for use in screening postmortem blood using Tecan® equipment. Pipetting was performed on Miniprep 75 using a 1:10 specimen dilution without any sample pretreatment. Plates were washed with a Columbus II plate washer and read using a Spectra II plate reader.

Pooled postmortem negative blood (as determined by ELISA and GC/MS) was used as a negative calibrator. A cut-off calibrator (2 ng/mL) and controls (1 ng/mL and 4 ng/mL) were prepared in-house by fortifying blood from the negative blood pool.

The within-run precision and linearity around the cutoff of the fentanyl assay was determined by assaying negative, 1, 2 and 4 ng/mL calibrators and controls (N=16) interspersed throughout a single plate. The assay demonstrated good precision and excellent separation as summarized in Table 1.

Table 1

Concentration	%CV	Average A/Ao	SD
Negative	5.27	97.81	5.15
1 ng/mL (low control)	6.73	47.04	3.16
2 ng/mL (cutoff)	7.64	26.42	2.02
4 ng/mL (high control)	11.72	14.84	1.74

Between-run precision was assessed by using the A/Ao obtained for the low and high controls that were assayed three and four times, respectively, in each ELISA batch. For the high control the CV was 13.2% (N=24). The low control had a CV of 13.3% (N=18). These CV's appeared to reflect the variation in ELISA assays and were not a result of pipetting imprecision as the Miniprep 75 demonstrated a CV of only 0.11% for the pipetting of the sample and diluent (N=64). ELISA assays typically demonstrated higher CV's than traditional immunoassays, however by calibrating using the mean of duplicate negative and cutoff calibrators, and due to the excellent separation around the cutoff calibrator, there were no failed controls on any batch ran.

Sensitivity, specificity, and efficiency were evaluated comparing the ELISA and GC/MS results with 314 blood specimens. These included a series of known positive samples and a series of sequential blood specimens analyzed in sequence as per routine casework. There were 225 true negatives and 88 true positives. The single false positive by ELISA was readily explained by the 1.9 ng/mL GC/MS concentration that was just below the cutoff. There were no false negatives. This resulted in excellent sensitivity (98.9%), specificity (99.6%), and efficiency (99.7%).

Fentanyl, ELISA, Method Evaluation

 ** Presenting Author*