K17 Screening Postmortem Whole Blood for Oxycodone by ELISA Response Ratios

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The goal of this presentation is to demonstrate screening of postmortem whole blood for oxycodone using the ratio of the oxycodone immunoassay response to the response for the specimen obtained with an opiate immunoassay.

A number of cases of diversion of OxyContin® and related prescription opiate narcotics for illegal use and abuse have been in the national press this past year. As a result of the popularity of these drugs, oxycodone may be increasingly encountered in driving, abuse and overdose cases. Oxycodone and related semisynthetic thebain derivatives may be missed by general opiate screens which are weakly cross-reactive with the C6-oxy opiates and by confirmation procedures which use GC/MS Selected Ion Monitoring (SIM) parameters set for morphine and codeine. Immunoassay response ratios can be used to related opiates. By dividing the response of a second oxycodonedirected immunoassay by the specimen response in a general opiate screen immunoassay, a relative immunoassay response ratio is obtained. Oxycodone-involved cases can be indicated by response ratios above an empirical cutoff threshold. This elevated ratio indicates which specimens should be confirmed for oxycodone, oxymorphone, hydrocodone and/or hydromorphone in addition to the confirmation for morphine and codeine.

Forty-eight specimens, which were negative for opiates, and one hundred sixty seven postmortem whole blood specimens, which were positive for opiates, including sixty-six specimens known to contain oxycodone, were assayed. Specimens were diluted 1:5 with assay buffer and analyzed by both the Neogen Oxymorphone/Oxycodone ELISA and the Neogen Opiate Group ELISA (Neogen Corporation, Lexington KY). Both immunoassays are microtiter plate-based ELISAs using horseradish peroxidase-labeled drug and anti-drug antibody immobilized to the microplate wells. Spiked whole blood calibration standards, specimens, the manufacturer's EIA standard, and negative and positive synthetic urine based controls were run on each plate. For the Opiates Group ELISA, standard concentrations were 0, 1, 5, 10, 20, 50 and 100 ng/ml morphine. For Oxymorphone/Oxycodone ELISA, the spiked standard concentrations were 0, 1, 5, 10, 20, 50 and 100 ng/ml oxymorphone. Diluted drug-enzyme conjugate was added to the microtiter plate wells and the mixture incubated at room temperature for 45 minutes. After incubation the plate was washed five times with wash buffer (phosphate buffer with Tween 20) using a Bio-Tek Elx50 Microplate Strip Washer (Bio-Tek Instruments, Highland park, Winoski, VT) to remove any unbound sample or drug-enzyme conjugate. K-Blue® substrate (tetramethylbenzidine (TMB) plus hydrogen peroxide) was added and after a 30-minute substrate incubation, the reaction was halted with the addition of Red Stop Solution (a non-acid peroxidase stop solution). The test was read using an Elx800 Universal Microplate Reader equipped with a 650 nm filter (Bio-Tek Instruments, Highland Park, Winoski, VT).

Calibration curves were plotted as log concentration vs the logit of the ratio of the mean absorbance at each concentration divided by the mean absorbance of the zero standard (B/B_o). The oxymorphone or morphine equivalents were estimated from the calibration curve using the ratio of the mean absorbance of the specimen to the mean absorbance of the zero standard.

The oxymorphone equivalents in ng/ml from the Oxymorphone/ Oxycodone ELISA were divided by the morphine equivalents in ng/ml from the Opiates ELISA to obtain an Oxycodone/Opiates Response Ratio. This ratio was compared to the GC/MS data for all specimens and for opiate positive specimens.

Sensitivity, the true positive rate, was calculated from the tally of true positives and false negatives determined by comparison of the GC/MS findings as: Sensitivity = TP/(TP + FN). Specificity was calculated as: Sensitivity = TN/(TN + FP). Because sensitivity and specificity are probabilities, the standard error (SE*p*) is equal to SE*p* = square root [p(1-p)/n]. Receiver Operating Curves (ROC) were obtained by plotting the sensitivity at each putative response ratio cutoff vs. (1 - specificity) at that cutoff value. The positive predictive value was calculated as fp/[fp + (1-f)(1-q)] where f is the prevalence in the population to be tested, p is the sensitivity and q is the specificity.

Specimens containing oxycodone produced large responses in the Oxycodone-directed immunoassay and positive but weaker responses in the general Opiate Group immunoassay. The median response ratio for oxycodone-containing specimens was 12.9; the mean was 33.7. The median response ratio for all opiate positive specimens **not** containing oxycodone was 0.055 and the mean was 7.3. ROC analysis was used to find an optimum response ratio cutoff value and to determine the probability that a specimen with this ratio would contain oxycodone or a related C6-oxy opiate. The optimum relative response ratio was 2.0. Specimens with a relative response ratio of 2.0 or higher had a greater than 50% probability (positive predictive value) of containing oxycodone. The sensitivity of the ELISA response ratio for the presence of oxycodone at a response ratio cutoff of 2.0 was 89.4% ± 3.8% and the specificity was 88.1% ± 3.2%.

The Neogen Opiates Group ELISA has a crossreactivity of 730% for codeine relative to 100% for morphine, 228% for hydrocodone, 35.6% for hydromorphine, 5.2% for oxycodone and 0.22% for oxymorphone. The Neogen Oxymorphone/Oxycodone ELISA has a cross-reactivity of 400% for oxycodone and 100% for oxymorphone, 30.8% for hydrocodone and 12.3% for hydromorphone; the crossreactivity with codeine is only 5.3% and for morphine 1.7%. The oxymorphone/oxycodone immunoassay has sufficient selectivity to identify OxyContin®- and other oxycodone-involved

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cases by using the ratio of the relative response to the Neogen Opiate Group ELISA result. Neither assay had a response within the calibration curve range with the negative whole blood specimens. However, some decomposed specimens caused false positive results with the ELISA assays. In conclusion, the Neogen Oxymorphone/Oxycodone ELISA can be used as a second immunoassay to identify which opiate-positive specimens should be confirmed for oxycodone.

Oxycodone, ELISA, Response Ratio

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