D7 Postmortem Quantitation of Insulin and C-peptide in Cases of Suspected Exogenous Insulin Administration

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After attending this presentation, attendees will be presented with convenient methods for insulin and C-peptide determinations in postmortem blood samples for the purpose of identifying exogenous insulin administration

This presentation will impact the forensic community and/or humanity by providing a validation and application of commercially available RIA kits for the quantitation of insulin and C-peptide in postmortem hemolyzed blood specimens. The kits provide technically easy to perform and economically cost effective methods to obtain reliable results in helping to establish the role of exogenous insulin in determining cause of death.

Homicide reports by exogenous insulin injection are rare. However, since insulin, and syringes to inject it, are readily available, and it is a potentially lethal drug, insulin should be given consideration in suspicious homicides. The ratio of insulin to C-peptide (insulin/Cpeptide) may be used to make a forensic diagnosis of exogenous insulin overdosage. Endogenous insulin is secreted in equal proportion with Cpeptide. Because C-peptide is more slowly cleared than insulin, the physiological insulin/C-peptide is less than one. Exogenous insulin will result in a ratio greater than one. Quantitation of insulin and C-peptide in a postmortem specimen can be cumbersome, and require large quantities to be present. Described below are simple and economical radioimmunoassays (RIAs) for quantitative determination of insulin and Cpeptide in postmortem blood specimens.

Commercially available radioimmunoassay (RIA) kits were obtained from Diagnostic Systems Laboratories, Inc. (Webster, TX). Both kits have received FDA clearance, and have been used in preclinical toxicological, clinical and diagnostic applications. The standard curve covers a range of insulin concentration from 1 to 300 uIU/mL, and 0.1 to 20 ng/mL for C-peptide. Both RIAs require only 100 uL of specimen for a single determination. Blood specimens were collected from three deceased individuals who had a medical history, which included diabetes and insulin therapy at the time of death. The specimens were grossly hemolyzed, even after centrifugation at 1000g. Duplicate aliquots of each specimen were stored frozen at –20 and 4 degrees C for three days. Because both RIAs were designed for determinations in non-hemolyzed serum and plasma, validation was required for a grossly hemolyzed postmortem blood specimen. Validation included adding known amounts of insulin and C-peptide standards to the hemolyzed specimens and performing assays for recovery and linearity studies.

Insulin standards ranging from 1 to 150 uIU/mL were added to the specimens and when assayed gave recoveries of 94 to 103%. Serial dilutions of 1:2, 1:4 and 1:8 of one specimen gave linearity from 75 to 120%. Only one specimen contained enough insulin to allow for linearity by dilution studies. All specimens were assayed in duplicate with results within 10% CVs. Using these procedures, the specimens were quantitatively assayed for insulin. Specimens stored at -20 degrees C had insulin levels of 88.5, 4.3 and undetectable (less than 1.5) uIU/mL. Specimens stored at 4 degrees C had lower insulin levels of 70.3, and less than 1uIU/mL in the remaining two specimens. Thus, freezing would be the preferred storage condition.

Recovery assays for C-peptide included additions ranging from 0.1 to 10 ng/mL, and gave recoveries of 52 to 98%. Linearity by dilution studies were not possible at this time because all three the specimen contained too little C-peptide. The CVs of duplicates were less than 10%. Cpeptide concentrations in the specimens from the three deceased cases were undetectable (less than 0.1), 0.25 and 0.1 ng/mL respectively. Specimens stored at 4 degrees C had undetectable C-peptide concentrations (less than 0.1 ng/mL). Again, freezing provided the best storage conditions.

In conclusion, the specimen with 88.5uIU/mL of insulin could be of exogenous source since the C-peptide was less than 0.1 ng/mL. In addition, the commercial assays have reliable performance for use with grossly hemolyzed postmortem specimens.

Insulin, C-Peptide, Postmortem

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