K13 Specificity Characteristics of Buprenorphine Immunoassays and Their Effects on the Correlation of Immunoassay Apparent Analyte Concentration With GC-MS Concentrations of Buprenorphine and Its Metabolites in Urine

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After attending this presentation, attendees will better comprehend the effect of immunoassay (IA) specificity on the commonly adapted 2-stage test methodology - IA and GC-MS for preliminary and confirmatory tests - for the analysis of buprenorphine (B) and its metabolites in urine specimens. This presentation will impact the forensic science community by reporting: (a) specificity characteristics of various commercially-available B IAs, and (b) the effect of these characteristics on the correlation of IA apparent B con- centration in clinical urine specimens to the concentrations of B and its metabolites (norbuprenorphine, NB; B glucuronide, BG, and NBG) as determined by GC-MS.

Performance characteristics of five B ELISA (Immunalysis, Neogen, Diagnostix, IDS-B, IDS-NB) and one analyzer-based (CEDIA by Microgenics) reagents currently available from commercial sources were studied to better understand their analytical parameters, including calibra- tion, cross-reacting characteristics, assay precisions and others. Information thereby derived were applied to the analysis of clinical urine specimens collected from heroin addicts under B "treatment" following required IRB protocols. Resulting IA *apparent* analyte concentrations were correlated against the concentrations of various metabolites as determined by GC-MS to better understand the effects of these IAs' specificity characteristics.

ELISA reagents studied were found to exhibit significant cross- reactivity toward BG in the order shown below: IDS-NB > Neogen > Diagnostic > IDS-B, while Immunalysis and Diagnostix reagents were found to significantly cross-react with NB. IDS-NB and Diagnostix reagents were also found to exhibit significant cross-reactivity toward NBG. The analyzer- based CEDIA reagent was found to show significant cross-reactivity toward BG and some cross-reactivity toward NBG and NB. Unlike other reagents studied, IDS-NB reagent was also found to exhibit significant cross-reactivity toward morphine, codeine, hydrocodone, hydromorphone, oxycodone, and naloxone.

With different cross-reactivity characteristics, apparent analyte concentrations derived from various IAs were found to correlate with the metabolites' concentrations (as determined by GC-MS) in different ways. For example, showing significant cross-reactivity toward BG, CEDIA reagent generated apparent B concentrations that do not correlate well with the concentrations of B (Figure 1A) or BG (Figure 1B) alone, but with significant correlation with the total concentration of B (Figure 1C).

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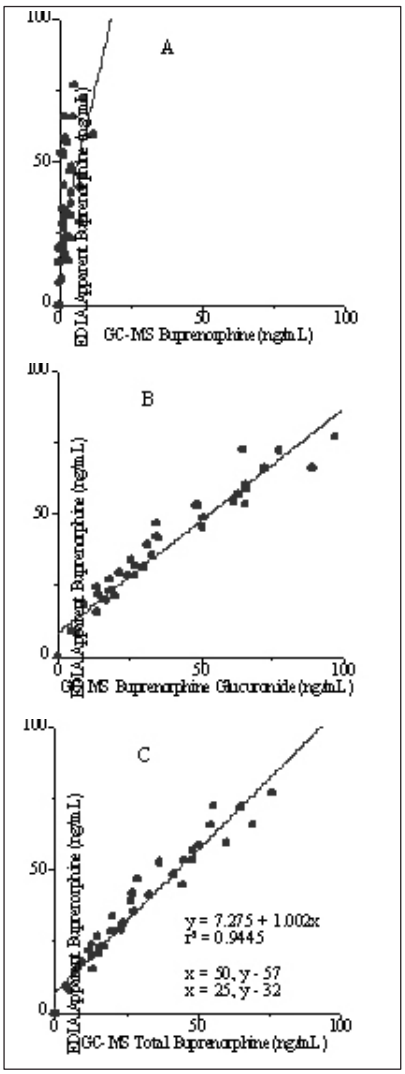

Figure 1. Correlation of CEDIA apparent buprenorphine concentration against GC-MS buprenorphine (A), buprenorphine glucuronide (B), and total buprenorphine (C) concentrations.

Buprenorphine, Glucuronide, Immunoassay

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