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### (2005-##) **Transferrin Extraction By Immunoaffinity Microcolumn Chromatography.**

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**Background:** Transferrin has traditionally aided clinicians in the diagnosis of iron-deficiency anemia. More recently, carbohydrate-deficient transferrin isoforms have been used for monitoring ethanol abuse. In this study, we used 200 microliter PhyTip™ immunoaffinity microcolumns (IAMC) containing 5 microliters of immobilized IgY (avian) antibody coated microbeads (Seppro™) to selectively remove transferrin from serum for further analysis.

**Objective:** Establish proof of principle by recovery of transferrin from serum using IAMC.

**Methods:** Serum transferrins were measured by the standard Beckman LX20 turbidimetric assay in 44 patients. Using these samples, serum transferrin was isolated by IAMC and concentrations were measured using a Beckman Access turbidimetric assay adapted for the LX20. Results for the two methods were used to determine the recovery of transferrin from serum using the IAMC. In addition, the potential for regeneration and reuse of the IAMC was evaluated. SDS-PAGE was used to verify transferrin isolation.

**Results:** Transferrin concentrations in three patient samples by the LX20 turbidimetric assay were 127, 229 and 331 mg/dL (n=6). Their recoveries measured by the modified Access assay after IAMC separation using new columns (n=6) were 62 +/- 5%, 80 +/- 4% and 78 +/- 6%, respectively. Additionally, recoveries when reusing the same columns 6 times were 79 +/- 16%, 86 +/- 17% and 85 +/- 15%, respectively. The isolation of >90% pure transferrin was confirmed using SDS-PAGE. Subsequently, transferrin was isolated in 44 patient samples using the IAMC technique. The comparison of transferrin by column capture versus standard serum turbidimetric assay yielded an average recovery of 91% and an overall  $R^2 = 0.78$  for serum values ranging from 60 to 330 mg/dL.

**Conclusion:** IAMCs can be used to specifically isolate transferrin from other serum proteins and could be applied to the measurement of other serum proteins using appropriate IgY antibody-coupled microbeads. Further investigation of this technique may have applications in such areas as the measurement of carbohydrate-deficient transferrin, a novel biomarker for ethanol abuse. While there was some randomness in the recovery obtained, there was no appreciable decline in transferrin recoveries after six uses for each of five different columns.