IP reagent was prepared from delipidated and stabilized goat anti-apo B antisera (LipoSep IP™, Sun Diagnostics). Dose-response studies indicated that equal volumes of sample and reagent completely precipitated all apo-B containing lipoproteins to 300 mg/dL with no effect on HDL. Incubation time (1 – 60 min), centrifugation speed (8,000-14,000 rpm) and centrifugation time (5 – 15 min) had little effect on results. For subsequent experiments 200 or 250 µL of reagent was added to an equal volume of sample and vortexed for 10 seconds, incubated for 10 minutes at room temperature, and centrifuged at 12,000 rpm for 10 minutes. Precision was assessed by IP of 10 replicates of a serum pool. HDL-C results for 25 serum samples with apo B concentrations from 45-150 mg/dL were determined by IP and dextran sulfate/MgCl2 precipitation. Specificity was determined by measuring apol A1 and B in 25 sera before and after IP. 

**RESULTS**

Total imprecision was 5.0%. Analytical imprecision, determined by combining sera samples after IP and measuring apo A1 in the supernatant pool 10 times, was 2.8%. By definition, the imprecision attributable to IP was 2.2%. HDL-C by IP (Y) gave excellent agreement to dextran sulfate/MgCl2 precipitation (Figure). The mean recovery of apo A1 after IP was 98.3% and 1.0%, respectively; all apo B results were < LOD. 

**CONCLUSIONS**

The IP reagent and protocol is a simple, effective and highly specific tool for isolating HDL particles in human serum.

**ABSTRACT**

**Background:** Immunoprecipitation (IP) with antisera provides the most specific method available for separation of lipoproteins. IP is simple to perform, does not alter lipoprotein particle composition and allows for more robust chemiluminescence than chemical methods. Here, we describe an IP reagent and procedure for isolation of HDL particles in human sera. **Methods:** IP reagent was delipidated and stabilized goat anti-apo B antisera. Dose-response studies indicated that equal volumes of sample and reagent completely precipitated all apo-B containing lipoproteins to 300 mg/dL with no effect on HDL. Incubation time (1 – 60 min), centrifugation speed (8,000-14,000 rpm) and centrifugation time (5 – 15 min) had little effect on results. For subsequent experiments 200 or 250 µL of reagent was added to an equal volume of sample and vortexed for 10 seconds, incubated for 10 minutes at RT, and centrifuged at 12,000 rpm for 10 minutes (Eppendorf microcentrifuge). Dextran sulfate precipitation was performed essentially as described by Warnick (5). 30 µL reagent was added to 300 µL sample, vortexed, incubated for 10 minutes at room temperature, and then centrifuged at 10,000 rpm for 5 min (Eppendorf microcentrifuge). Cholesterol (Wako Chemicals USA) and apol A1 and B (laboratory developed tests) were measured in supernatants using a Cobas Fara II analyzer. Precision was assessed by immunoprecipitation of 10 replicates of a serum pool. HDL-C results for 25 normolipidemic serum samples were compared after IP and dextran sulfate/MgCl2 precipitation. Robustness of IP and chemical precipitation was determined with 32 turbid and/or high triglyceride specimens to compare the frequency of incomplete precipitation.

**INTRODUCTION**

HDL particles play an important role in reverse cholesterol transport (RCT), the movement of cholesterol from peripheral tissue back to the liver for excretion, and although RCT is presumed to be the primary mechanism for HDL’s protective effect, HDL also have anti-inflammatory, and antithrombotic activities, and appear to health endothelial function (1). Characterization of high density lipoproteins (HDL) is currently the focus of intense research. The concept of “dysfunctional HDL”, HDL particles that are somehow altered and no longer protective against CHD, is relatively new. Clinicians have long noticed the paradox that some individuals with very high HDL cholesterol concentrations develop CHD in the absence of obvious risk factors. Recent data has implicated certain proteins associated with these dysfunctional HDL (2). In addition to HDL function, new classes of pharmaceuticals, including CETP inhibitors, are being developed to raise HDL concentrations. The challenge for these researchers is to isolate HDL particles for measurement of cholesterol and other components that may explain function or better monitor therapeutic efficacy. 

Traditional approaches such as gel electrophoresis and ultracentrifugation are labor intensive, technically demanding, and may alter particle composition and structure; chemical precipitation methods may not completely remove apo-B containing lipoproteins or adequately capture all HDL subfractions; and homogeneous methods for HDL cholesterol measurement have proven to be inadequate in patients with certain lipoprotein abnormalities. Immunoprecipitation shares the positive attributes of chemical precipitation methods such as ease of use, but also allows quantitative separation of HDL particles from apo B lipoproteins, without altering lipoprotein particle composition.