Immunoprecipitation of Apo B-containing Lipoproteins for Isolation of HDL Particles and Comparison to Dextran Sulfate/Magnesium Chloride Precipitation

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ABSTRACT

Background: Immunoprecipitation (IP) of non-HDL particles with antisera provides the simplest and most specific method available for separation of HDL. IP is simple, does not alter lipoprotein composition and allows for more robust precipitation than chemical methods. Objective: To compare LipoSep™ IP reagent (Sun Diagnostics, New Gloucester, ME) with the dextran sulfate/$MgCl_2$ designated comparison method (Kimberly, Clin Chem 1999; 45:1803). Methods: Dose-response studies indicated that equal volumes of sample and IP reagent precipitated all apoB to 300 mg/dL without coprecipitation of HDL (Nguyen, Clin Chem 2013; 59 Supplement: A268). For these experiments 200µL of IP reagent was added to an equal volume of serum, vortexed for 10 seconds, incubated for 10 minutes at room temperature, and microcentrifuged at 12,000 rpm for 10 minutes. HDL-C results for 118 serum samples with apoB concentrations from 16-211 mg/dL were determined by IP and by dextran sulfate/$MgCl_2$ precipitation. Specificity was determined by measuring apoAI and apoB before and after IP. Efficiency was assessed by comparing the ability of both reagents to form a pellet with hypertriglyceridemic samples. Cholesterol was measured with enzymatic reagents (Wako Diagnostics, Richmond, VA) and apos AI and B were measured with laboratory developed immunoturbidimetric assays. All measurements were made on a Cobas Fara II analyzer. Results: HDL-C measured in the supernatant after IP (Y) gave excellent agreement to dextran sulfate/$MgCl_2$ precipitation (X) with a slope of 1.01, an intercept of 2.7 mg/dL, and a correlation of 0.99. However, dextran sulfate/MgCl2 precipitation failed in most samples with moderate to elevated triglycerides. At triglyceride concentrations from 290 to 2,091mg/dL the initial success rate was 65% for IP, while dextran sulfate/$MgCl_2$ successfully precipitated only one of 79 samples (1.3%). Success rate on repeat with additional reagent and/or sample dilution gave a success rate of 81% for IP and 42% for dextran sulfate/$MgCl_2$. The mean recovery of apos AI and B after IP was 95.4% and 0.7%, respectively, showing specificity for apo B-containing lipoproteins. Conclusion: The IP reagent and protocol is a simple, effective and highly specific tool for isolating HDL particles in human serum, and is an excellent tool for researchers seeking to quantitatively isolate HDL from other lipoproteins. Unlike chemical precipitation, immunoprecipitation is effective with high triglyceride samples.

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INTRODUCTION

HDL particles play an important role in reverse cholesterol transport (RCT) and protection from coronary heart disease (CHD). Although RCT is presumed to be the primary mechanism for HDL's protective effect, HDL also have anti-inflammatory, antioxidant, and antithrombotic activities, and appear to promote healthy 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 quantitatively isolate HDL particles for measurement of cholesterol and other components that may explain function or better monitor therapeutic efficacy***.

Traditional approaches such as 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 subclasses; 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.***

METHODS

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 (3). For these experiments 200 µL of reagent was added to an equal volume of sample and vortexed for 10 seconds, incubated for 10 min at RT, and centrifuged at 12,000 rpm for 10 min (Eppendorf microcentrifuge). Dextran sulfate precipitation was performed as described by Kimberly (4); 30 µL reagent was added to 300 µL sample, vortexed, incubated for 10 min at room temperature, and then centrifuged at 10,000 rpms for 5 min (Eppendorf microcentrifuge). Cholesterol (Wako Chemicals USA) and apos AI and B (laboratory developed tests) were measured in supernatants using a Cobas Fara II analyzer. HDL-C results for 118 serum samples were compared after IP and dextran sulfate/$MgCl_2$ precipitation. Robustness of IP and chemical precipitation was determined with turbid and/or high triglyceride specimens to compare the frequency of incomplete precipitation.

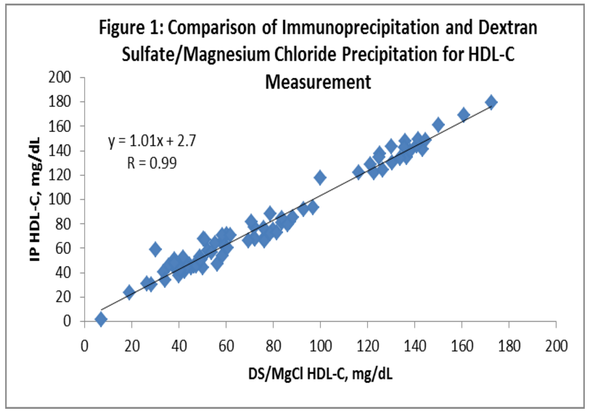
RESULTS

HDL-C by IP gave excellent agreement to dextran sulfate/$MgCl_2$ precipitation (Figure1). The mean recovery of apo AI and B in 25 serum samples after IP was 95.4% and 0.7%, respectively; all apo B results were < LOD of the assay. Apo AI recovery was essentially 100% with mean difference due to assay variability. IP successfully removed all apo B-containing lipoproteins in samples with triglycerides up to about 2000 mg/dL, while dextran sulfate/magnesium chloride was successful only up to about 500 mg/dL triglycerides, and only after repeat testing with additional reagent or sample dilution (Table 1). Where DS/$MgCl_2$ precipitation was successful, agreement by IP was very good. However, IP was much more robust in isolating HDL particles in high triglyceride samples.

Table 1: Successful Precipitation and Measurement in Samples with Elevated Triglycerides; Immunoprecipitation vs. Chemical Precipitation

TRIG, mg/dL	N	IP 1st Attempt	IP 2nd Attempt	DS 1st Attempt	DS 2nd Attempt
250-399	11	100%	---	18.2%	100%
400-699	9	77.8%	100%	0%	55.6%
700-999	14	85.7%	100%	0%	14.3%
≥1000	18	22.2%	61.1%	5.6%	16.7%
ALL	52	65.4%	86.5%	5.8%	40.4%

Key: 1st Attempt: Usual procedure; 2nd Attempt: Additional reagent and/or sample dilution prior to precipitation. IP: Immunoprecipitation reagent; DS: Dextran sulfate/magnesium chloride reagent; TRIG: Triglycerides.



DISCUSSION

Chemical precipitation methods are generally effective in separating HDL particles from apo B-containing lipoproteins. Typically, polyanions such as heparin, dextran sulfate, and sodium phosphotungstate are used with a divalent cation, such as magnesium or manganese. The dextran sulfate magnesium chloride precipitation method, using dextran sulfate with an approximate molecular weight of 50,000 is the most popular precipitation method in the US, and is recognized as an important component of the designated comparison method (DCM) for HDL cholesterol measurement (4).

Unfortunately, with chemical methods, triglyceride-rich lipoproteins (TRL) may not completely precipitate, as evidenced by turbidity in the supernatant. According to Warnick and colleagues, chemical precipitation methods may slightly overestimate HDL cholesterol due to incomplete precipitation of VLDL and LDL (5). All methods fail with very high triglycerides presumably because the higher densities of the TRLs make sedimentation by centrifugation difficult. In one study, the percentage of samples requiring additional treatment because of incomplete precipitation was 4%, 7.5%, 10%, 11%, and 12% for PEG (10%), dextran sulfate/magnesium chloride, heparin/manganese, and PEG (7.5%), respectively (5). Warnick et al (3) determined that both dextran sulfate/magnesium and heparin/manganese left small amounts of apo B in the supernate, while precipitating small amounts of HDL. Dextran sulfate precipitated slightly more apo B-containing lipoproteins than heparin, but also precipitated slightly more HDL.

Monospecific antibodies directed against apolipoproteins are the most specific method available for the separation of lipoproteins. Puchols and colleagues compared ultracentrifugation and chemical precipitation methods to immunoprecipitation for the isolation of HDL and reported that only immunoprecipitation completely separated apo AI and Apo B containing particles (6); Chemical precipitation and ultracentrifugation failed to separate 4% to 20% of HDL particles (6). Similar results were reported by Heuck and colleagues with immunoprecipitation; no beta or pre-beta lipoproteins were present in the supernatant, with all alpha lipoproteins remaining (7).

Our data are consistent with these previous studies showing that immunoprecipitation is specific in isolating HDL particles, and more robust in sedimenting triglyceride-rich lipoproteins. The availability of a validated, commercially available immunoprecipitation reagent will prove useful to clinicians and researchers with a need to accurately separate HDL particles.

CONCLUSIONS

Apolipoprotein recognition by antibodies can be used as a highly specific tool for identifying and characterizing lipoprotein subclasses. One can easily see the advantages of immunoprecipitation to isolate HDL for measurement of cholesterol or other HDL components. These data clearly show that immunoprecipitation is more robust than chemical precipitation in clearing triglyceride-rich lipoproteins. Because of the specificity of anti-apo B antibodies, HDL particles will not co-precipitate with apo B-containing lipoproteins, which may be an issue with chemical precipitation methods.