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

John H. Contois, Andre L. Albert, and Rae-Anne Nguyen; Sun Diagnostics, New Gloucester, ME

ABSTRACT

Background: Immunoprecipitation (IP) of non-HDL particles with anti-apoB provides the simplest and most specific method for separating HDL. IP is simple, does not alter lipoprotein composition and allows for more robust precipitation than chemical methods. Objective: To compare Lipoprep™ IP reagent (Sun Diagnostics, New Gloucester, ME) with the dextran sulfate/MgCl2 designated comparison method (Kimberly, Clin Chem 1999; 45:1803). Methods: Dose-response studies indicated that equal volumes of sample and IP reagent were required to precipitate 100% of HDL without coprecipitation of HDL. Nguyen, Clin Chem 2013; 59 Supplement: A268). For these experiments, 200 µL of IP reagent was added to an equal volume of sample in two 10 seconds, incubated for 10 min at room temperature, and then centrifuged at 10,000 rpm for 5 min. The mean recovery of apo AI and HDL was 95.4% and 0.7%, respectively; all apo B results were <LOD of the assay. APC recovery was essentially 100% with mean difference due to assay variability. IP successfully removed all apo AI-containing lipoproteins in samples with triglycerides up to about 2000 mg/dL, while dextran sulfate/magnesium chloride precipitation was successful only up to about 500 mg/dL triglycerides, and only after retesting with additional reagent or sample dilution (Table 1). Where DS/MgCl2 precipitation was successful, agreement by IP was very good. However, IP was much more robust in isolating HDL particles in high triglyceride samples.

DISCUSSION

Chemical precipitation methods are generally effective in separating HDL particles from apo B-containing lipoproteins. Typically, poly-anion agents 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 (40%), 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 supernatant, while precipitating small amounts of HDL. Dextran sulfate precipitated slightly more apo B-containing lipoproteins than heparin, but also precipitated slightly more HDL.

Conclusively, immunoprecipitation with anti-apoB antibodies is the most specific method available for the separation of lipoproteins. Pachols 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 6% of lipoprotein particles (6). Similar results were reported by Heuck and colleagues with immunoprecipitation; no beta or prebeta 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.

REFERENCES


INTRODUCTION

HDL particles play an important role in reverse cholesterol transport (RCT) and prevention of arterial 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.

METHODS

Immunoprecipitation was performed from delipidated and stabilized goat anti-apo B antiserum (LipoSep IP™, Sun Diagnostics). Dose-response studies indicated that equal volumes of sample and reagent completely precipitated all apo B-containing lipoproteins. 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.

RESULTS

HDL by IP gave excellent agreement to dextran sulfate/MgCl2 precipitation (Figure 1). The mean recovery of apo AI and HDL in 25 serum samples after IP was 95.4% and 0.7%, respectively; all apo B results were <LOD of the assay. APC recovery was essentially 100% with mean difference due to assay variability. IP successfully removed all apo AI-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 retesting with additional reagent or sample dilution (Table 1). Where DS/MgCl2 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

<table>
<thead>
<tr>
<th>TRIG, mg/dL</th>
<th>N</th>
<th>IP 1st Attempt</th>
<th>IP 2nd Attempt</th>
<th>DS 1st Attempt</th>
<th>DS 2nd Attempt</th>
</tr>
</thead>
<tbody>
<tr>
<td>250-999</td>
<td>11</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>400-699</td>
<td>9</td>
<td>77.8%</td>
<td>100%</td>
<td>0%</td>
<td>55.6%</td>
</tr>
<tr>
<td>700-999</td>
<td>14</td>
<td>85.7%</td>
<td>100%</td>
<td>0%</td>
<td>14.3%</td>
</tr>
<tr>
<td>≥1000</td>
<td>18</td>
<td>65.4%</td>
<td>86.5%</td>
<td>5.8%</td>
<td>40.4%</td>
</tr>
</tbody>
</table>

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.

Figure 1: Comparison of Immunoprecipitation and Dextran Sulfate/Magnesium Chloride Precipitation for HDL-C Measurement

CONCLUSIONS

Apolipoprotein recognition by antibodies can be used as a highly specific tool for identifying and characterizing lipoprotein subclasses. One can easily study the advantages of immunoprecipitation over the traditional chemical precipitation (CP) method for measuring cholesterol or other HDL components. These data clearly show that immunoprecipitation is more robust than chemical precipitation in measuring 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.

Figure 2: Comparison of Immunoprecipitation and Dextran Sulfate/Magnesium Chloride Precipitation for HDL-C Measurement