INTENDED USE
NS Biotech HDL-precipitating reagent is intended for the in vitro quantitative separation of High-Density Lipoproteins (HDL) in serum on manual systems.

CLINICAL SIGNIFICANCE
Plasma lipoproteins are spherical particles containing varying amounts of cholesterol, triglycerides, phospholipids and proteins. The phospholipids, free cholesterol, and protein constitute the outer surface of the lipoprotein particle, while the inner core contains mostly esterified cholesterol and triglycerides. The particles serve to solubilize and transport cholesterol and triglycerides in the blood stream. The relative proportions of protein and lipid determine the density of these lipoproteins, and provide a basis on which to begin their classification. These classes are: chylomicron, very low density lipoprotein (VLDL), low density lipoprotein (LDL), and high-density lipoprotein (HDL). Numerous clinical studies have shown that the different density lipoprotein classes have very distinct and varied effects on coronary heart disease risk.

ASSAY PRINCIPLE
The reference procedure for separation of the different lipoprotein density classes is density adjusted ultra centrifugation, which is not a practical technique for routine laboratory use. Widely used alternatives are based on polyanion precipitation techniques: polyanions such as heparin, dextran sulphate and phosphotungstinate, bind to positively charged Apoprotein B-containing lipoproteins which are then linked by the use of a divalent cation (manganese, magnesium or calcium) to form a precipitate. The NS Biotech HDL-precipitating method utilizes the well established precipitating properties of phosphotungstic acid to precipitate non HDL-cholesterol. This precipitation technique is the most frequently used method for HDL procedures. The remaining cholesterol in the supernatant, HDL-cholesterol, can then be measured using NS Biotech cholesterol reagent kit.

EXPECTED VALUES

| Males 6 | Good prognosis > 55 mg/dl (>1.4 mmol/L) |
| Standard risk 35 – 55 mg/dl (0.9 -1.4 mmol/L) |
| Risk indicator < 35 mg/dl (<0.9 mmol/l) |
| Females 6 | Good prognosis > 65 mg/dl (>1.7 mmol/L) |
| Standard risk 45 – 65 mg/dl (1.2 -1.7 mmol/L) |
| Risk indicator < 45 mg/dl (<1.2 mmol/l) |

REAGENTS
- R1 HDL-Cholesterol standard 50 mg/dl
- R2 Precipitating reagent
  - Phosphotungstic acid 40.0 g/l
  - MgCl2 100 g/l
  - Buffer pH 6.2
- Additional reagent required but not provided NS Biotech cholesterol reagent

- Reagent Preparation & Stability
All reagents are stable up to the expiry date given on label when stored at room temperature protected from light.

SPECIMEN
- Use fresh non-hemolyzed serum.
- EDTA and heparinized plasma are the only accepted anticoagulants.
- A specimen drawn from a fasting patient is preferred, although non-fasting specimens are considered acceptable.

Specimen Preparation & Stability
- Serum separated from the blood clot within 4 hours at room temperature.
- Store plasma at 4°C prior to analysis. EDTA plasma has the advantage that lipoproteins have enhanced stability during storage at 4°C.
- Specimens should preferably be analyzed on the day of collection.
- HDL-cholesterol is stable in specimen for 4 days at 4°C or 7-14 days at -20°C. HDL-cholesterol decreased significantly, but the decrease is not clinically relevant.

Specimen Preparation & Stability
- HDL Separation
  1. Dispense 500 µl of specimen at room temperature in to an appropriately labeled test tube.
  2. Add 50 µl of precipitating reagent (R2). Mix well.
  3. Incubate the test tube at room temperature for 10 minutes prior to centrifugation.
  4. Centrifuge for 10 minutes at full speed (at least 1000 x g). Don’t use warm or hot centrifuge.
  5. Separate the supernatant, which contain the HDL, from the precipitate.

- HDL Cholesterol Analysis
The supernatant can be assayed using NS Biotech cholesterol reagent. The following procedure is for manual instrumentation but can be adapted to most automated instruments. Specific instrument applications are available upon request.

| Wavelength | 500 - 550 nm |
| Cuvette | 1 cm light path |
| Temperature | 20-25 or 37 °C |
| Zero adjustment | against reagent blank |

<table>
<thead>
<tr>
<th>Blank</th>
<th>Standard</th>
<th>Specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol reagent</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Standard</td>
<td>50 µl</td>
<td>50 µl</td>
</tr>
<tr>
<td>Specimen</td>
<td>......</td>
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</tbody>
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Mix, incubate for 5 minute at 37°C or 10 minute at 20-25°C. Measure the absorbance of specimen (A specimen) and standard (A standard) against reagent blank.

The color is stable for 60 minutes.

CALCULATION
Calculate the HDL-cholesterol concentration by using the following formulae:

HDL-Cholesterol Concentration =

Absorbance of Specimen x Standard value
Absorbance of Standard

- Unit conversion
mg/dl x 0.0259 = mmol/l
A reference range for HDL-cholesterol is not sufficient for interpretation of results. HDL-cholesterol is used in the Friedewald formula\textsuperscript{10} to evaluate the risk of developing coronary heart disease.

\[
\text{LDL-cholesterol (mg/dl)} = \text{Total cholesterol} - \text{HDL-cholesterol} - \left(\frac{\text{triglycerides}}{5}\right).
\]

\[
\text{LDL-cholesterol (mmol/L)} = \text{Total cholesterol} - \text{HDL-cholesterol} - \left(\frac{\text{triglycerides}}{2.181}\right).
\]

This formula is usually valid for triglycerides concentrations up to 400 mg/dl (4.5 mmol/L).

Recommendations of the laboratory Standardization Panel of the National Cholesterol Education Program\textsuperscript{11}.

### Desirable risk
- Total cholesterol: <200 mg/dl (<5.2 mmol/L)
- LDL-cholesterol: <130 mg/dl (<3.4 mmol/L)

### Borderline high risk
- Total cholesterol: 200-239 mg/dl (5.2-6.2 mmol/L)
- LDL-cholesterol: 130-159 mg/dl (3.4-4.1 mmol/L)

### High risk
- Total cholesterol: >240 mg/dl (>6.2 mmol/L)
- LDL-cholesterol: >160 mg/dl (>4.1 mmol/L)

### LINEARITY

When run as recommended, the assay is linear up to 150 mg/dl. Specimens with values above 150.0 mg/dl should be diluted with 0.9% NaCl solution and reassayed. Multiply the result by the dilution factor.

### SENSTIVITY

The sensitivity is defined as the change of analytical response per unit change in analyte concentration at a pathlength of 1 cm. When run as recommended the sensitivity of this assay is 3.0 mg/dl.

### QUALITY CONTROL

It is recommended that controls (normal and abnormal) be included in:
- Each set of assays, or
- At least once a shift, or
- When a new bottle of reagent is used, or
- After preventive maintenance is performed or a clinical component is replaced.

Commercially available control material with established HDL-cholesterol values may be routinely used for quality control. Failure to obtain the proper range of values in the assay of control material may indicate:
- Reagent deterioration,
- Instrument malfunction, or
- Procedure errors.

The following corrective actions are recommended in such situations:
- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results on fresh control material still remain outside the limits, then repeat the test with fresh reagent.
- If results are still out of control, contact NS Biotec Technical Services.

### INTERFERING SUBSTANCES

- **Haemolysis:**
  Haemoglobin levels higher than 7.5 g/l decrease the apparent HDL-cholesterol concentration significantly.
- **Icterus:**
  Bilirubin levels higher than 23.4 mg/dl decrease the apparent HDL-cholesterol concentration significantly.
- **Lipemia:**
  Intralipid levels higher than 1000 mg/dl decrease the apparent HDL-cholesterol concentration significantly.
- **Anticoagulants:**
  The only acceptable anticoagulants are heparin and EDTA.
- **Others:**
  Ascorbic acid level higher than 100 mg/dl decrease the apparent HDL-cholesterol concentration significantly. In rare cases, elevated immunoglobulin concentrations can lead to falsely increase HDL-cholesterol result.

### WARNING & PRECAUTIONS

- NS Biotec HDL–precipitating reagent is for in vitro diagnostic use only. Normal precautions exercised in handling laboratory reagents should be followed.
- Don’t use HDL-precipitating reagent if it has become cloudy. Specimen with more than 1700 mg/dl triglycerides should be diluted with 0.9% NaCl solution and reassayed. Multiply the result by the dilution factor.

### BIBLIOGRAPHY