INTENDED USE
HDL-Cholesterol reagent is intended for the in vitro quantitative determination of High Density Lipoprotein Cholesterol in human serum or plasma. The reagent can assist in the diagnosis and treatment of patients at risk for developing coronary heart disease. Low HDL cholesterol is related to the high risk of coronary disease.

CLINICAL SIGNIFICANCE
High-density lipoproteins (HDL) compose one of the major classes of plasma lipoproteins. They are synthesized in the liver as complexes of apolipoprotein and phospholipids and are capable of picking up cholesterol and carrying it from arteries to the liver, where the cholesterol is converted to bile acids and excreted into the intestine. An inverse relationship between HDL-cholesterol (HDL-C) levels in serum and the incidence/prevalence of coronary heart disease (CHD) has been demonstrated in a number of epidemiological studies. The importance of HDL-C as a risk factor for CHD is now recognized. Accurate measurement of HDL-C is of vital importance when assessing patient's risk for CHD.

ASSAY PRINCIPLE
The assay is based on a modified polyvinyl sulfonylic acid (PVS) and polyethylene-glycol-methyl ether (PEGME) coupled classic precipitation method with the improvements in using optimized quantities of PVS/PEGME ad selected detergents. LDL, VLDL, and chylomicron (CM) react with PVS and PEGME and the reaction results in inaccessibility of LDL, VLDL and CM by cholesterol oxidase (CHOD) and cholesterol esterase (CHER). The enzymes selectively react with HDL to produce H₂O₂, which is detected through a Trinder reaction.

HDL + LDL + VLDL + CM
HDL + CHOD + CHER → Fatty Acid + H₂O₂
Peroxidase
H₂O₂ + 4-AA + TODB → Quinone + 5 H₂O
(λ₅₆₀nm)

MATERIALS REQUIRED BUT NOT PROVIDED
Any instrument with temperature control of 37 ± 0.5°C that is capable of reading absorbance accurately at 600 nm may be used. Controls for validating the performance of the HDL-Cholesterol reagents are sold separately (DZ129A-CON). Saline for diluting serum samples and for use as the zero calibrator is not provided.

REAGENT COMPOSITION
Reagent 1 (R₁)
MES buffer (pH 6.5)
TODB N, N-Bis (4-sulfobutyl)-3-methylalanine
Polyvinyl sulfonylic acid
Polyethylene-glycol-methyl ether
MgCl₂
Detergent
EDTA

Reagent 2 (R₂)
MES buffer (pH 6.5)
Cholesterol esterase
Cholesterol oxidase
Peroxidase
4-aminoantipyrine
Detergent

REAGENT PREPARATION
HDL-Cholesterol Assay Reagent (R₁, R₂) are liquid stable, ready-to-use reagents. The calibrator is provided in lyophilized form and must be reconstituted with saline before use.

REAGENT STABILITY AND STORAGE
Unopened reagents are stable until the expiration date printed on the outer box when stored at 2-8°C. Reagent on-board stability is at least 60 days. The reagent solutions should be clear. If turbid, the reagents may have deteriorated.

SPECIMEN COLLECTION AND HANDLING
Use fresh fasting patient serum and plasma samples (EDTA, Citrate, Li Heparin). If samples contain HDL cholesterol greater than 184.8 mg/dL, they should be diluted with saline.

PRECAUTIONS
For in vitro diagnostic use only.
Specimens containing human sourced materials should be handled as if potentially infectious using safe laboratory procedures, such as those outlined in Biosafety in Microbiological and Biomedical Laboratories (HHS Publication Number [CDC] 93-8395). As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient. Avoid ingestion and contact with skin and eyes. See Material Safety Data Sheet. Reagents are light-sensitive. Do not let bottles remain open. Keep container tightly closed. Do not use the reagents after the expiration date labeled on the outer box.

ASSAY PROCEDURES
Test Scheme for Chemistry Analyzers

<table>
<thead>
<tr>
<th>Time</th>
<th>R₁: 225 µL</th>
<th>R₂: 75 µL</th>
<th>Read A₁ at 600nm</th>
<th>Read A₂ at 600 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 min</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>10 min</td>
<td></td>
<td></td>
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</tbody>
</table>

Application sheets for use of RBI HDL-Cholesterol Reagents Assay on automated clinical chemistry analyzers are available upon request.

CALIBRATION
HDL-Cholesterol calibrator (DZ129A-Cal) should be used to calibrate the RBI HDL-Cholesterol Reagent. 0.9% saline should be used as a zero point calibrator. HDL-Cholesterol Calibrators are provided in lyophilized form and are stable until their expiration date when stored at 2-8°C.

Reconstitute contents with distilled water per instructions on vials and mix gently. Let vials equilibrate to room temperature for 30 minutes before use. Reconstituted calibrator is stable for 7 days when capped tightly and stored at 2-8°C. Calibration curve is stable for at least 14 days.

QUALITY CONTROL
We recommend that each laboratory uses HDL-Cholesterol controls to validate the performance HDL-Cholesterol reagent. A set of low, medium and high HDL-Cholesterol controls is available from RBI (Cat. # DZ129A-CON). If the results from the controls fall outside acceptable limits, as determined by their assigned values, the test should not be performed.

RESULTS
Sample Calculations

\[
ΔA = A_0 - A_1
\]

Concentration of HDL –Cholesterol in serum:

\[
ΔA\text{ sample} - ΔA\text{ blank} \times \text{standard} = ΔA\text{ standard} - ΔA\text{ blank}
\]

HDL-Cholesterol concentration is expressed as mg/dL.

To convert from conventional units to S.I. units, multiply the conventional units by 0.02586.¹⁰

\[
\text{mg/dL} \times 0.02586 = \text{mmol/L} \text{ HDL-Cholesterol}
\]

Results (in mg/dL) are printed out automatically by Hitachi 917. For other instruments, refer to the operator manual for printout instructions.
**REFERENCE RANGE**

The expected values for serum HDL Cholesterol are as follows: Less than 40 mg/dL – A major risk factor for heart disease.

40 to 59 mg/dL – The higher your HDL, the better.

60 mg/dL and above – An HDL of 60 mg/dL and above is considered protective against heart disease. Each laboratory must establish its own range of expected values.

**LIMITATIONS**

A sample with an HDL-Cholesterol level exceeding the linearity limit should be diluted with 0.9% saline and re-assayed incorporating the dilution factor in the calculation of the value. Do not freeze the reagents. Store the reagent at 2-8°C. Do not freeze the reagents.

**PERFORMANCE CHARACTERISTICS**

All performance characteristics were determined at Resolution Biomedical, Inc. using a Hitachi 917 chemistry analyzer.

**LIMIT OF BLANK**

The limit of blank (LOB) of the RBI HDL-Cholesterol Assay was determined as follows: HDL zero calibrator was tested 12 replicates on Hitachi 917. The LOB = mean + 3SD = 1.06 mg/dL

**ACCURACY**

The performance of this assay was compared with the performance of a legally marketed HDL-Cholesterol assay using serum samples. Eighty-four serum samples ranging from 5.7 to 189.3 mg/dL gave a correlation coefficient of 0.987. Linear regression analysis gave the following equation:

\[ \text{method} = 1.048(\text{reference method}) - 4.69 \text{mg/dL} \]

**PRECISION STUDIES**

The precision of the RBI HDL-Cholesterol Reagent was evaluated according to Clinical Laboratory Standards Institute (CLSI) EP5-A guideline.

In the study, three serum specimens containing 30, 55 and 90 mg/dL HDL-Cholesterol were tested on Hitachi 917 with 2 runs per day with duplicates over 20 working days. This method has not been tested or certified by the Cholesterol Reference Method Laboratory Network (CRMLN).

**Within-Run Precision**

<table>
<thead>
<tr>
<th>Level 1</th>
<th>Level 2</th>
<th>Level 3</th>
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</thead>
<tbody>
<tr>
<td>30 mg/dL</td>
<td>55 mg/dL</td>
<td>90 mg/dL</td>
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<table>
<thead>
<tr>
<th>Number of Data Points</th>
<th>Mean (µM)</th>
<th>SD (µM)</th>
<th>CV%</th>
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<tbody>
<tr>
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<tr>
<td>80</td>
<td>90.56</td>
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</table>

**Within-Laboratory Precision (S.)**

<table>
<thead>
<tr>
<th>Level 1</th>
<th>Level 2</th>
<th>Level 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 mg/dL</td>
<td>55 mg/dL</td>
<td>90 mg/dL</td>
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<thead>
<tr>
<th>Number of Data Points</th>
<th>Mean (µM)</th>
<th>SD (µM)</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
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<td>0.65</td>
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</tr>
<tr>
<td>80</td>
<td>53.07</td>
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<td>2.3</td>
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<tr>
<td>80</td>
<td>90.56</td>
<td>2.02</td>
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</tr>
</tbody>
</table>

**LINEARITY**

The linearity range of the assay is from 1.06 to 184.8 mg/dL in serum. Results below 1.06 mg/dL are invalid. Results that exceed 184.8 mg/dL should be diluted with saline and retested.

**INTERFERENCE**

The following substances normally present in serum produced less than 10% deviation at the listed concentrations: Triglycerides at 1000 mg/dL, ascorbic acid at 10 mM, Bilirubin at 40 mg/dL, Bilirubin Conjugated at 40 mg/dL, and Hemoglobin at 100 mg/dL.

**REFERENCES**


Castelli, W.P. et al., HDL Cholesterol and other lipids in coronary heart disease. Circulation, 55;767 (1977)


Williams, P., et al., High density lipoprotein and coronary risk factor, Lancet, 1;72don (1979)


NATIONAL INSTITUTES OF HEALTH publication No. 93-3095, September, (1993)


**Within-Run Precision**

ClearChem Diagnostics