**HDL CHOLESTEROL**

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

**REFERENCE VALUES**

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low risk</td>
<td>&gt; 50 mg/dL</td>
<td>&gt; 60 mg/dL</td>
</tr>
<tr>
<td>Normal</td>
<td>35 – 50 mg/dL</td>
<td>45 – 60 mg/dL</td>
</tr>
<tr>
<td>High risk</td>
<td>&lt; 35 mg/dL</td>
<td>&lt; 45 mg/dL</td>
</tr>
</tbody>
</table>

These values are for orientation purpose; each laboratory should establish its own reference range.

**PERFORMANCE CHARACTERISTICS**

**Measuring range:** from detection limit of 2.5 mg/dL to linearity limit of 200 mg/dL.

If the results obtained were greater than linearity limit, dilute the sample 1:2 with NaCl 9 g/L and multiply the result by 2.

**Precision:**

<table>
<thead>
<tr>
<th></th>
<th>Mean (mg/dL)</th>
<th>SD</th>
<th>0.3</th>
<th>0.2</th>
<th>0.7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>32.9</td>
<td>0.3</td>
<td>0.2</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>28.2</td>
<td>0.4</td>
<td>0.7</td>
<td>1.1</td>
<td></td>
</tr>
</tbody>
</table>

**Sensitivity:** 1 mg/dL = 0.0016 A.

**Accuracy:** Results obtained using BSM reagents (y) did not show systematic differences when compared with other commercial reagents (x).

The results obtained using 50 samples were the following:

- Correlation coefficient (r): 0.996.
- Regression equation: y = 0.98 + 3.42 mg/dL.
- The results of the performance characteristics depend on the analyzer used.

**INTERFERENCES**

No interferences were observed to bilirubin T. and D. up to 50 mg/dL, hemoglobin up to 1000 mg/dL or lipemia up to 1800 mg/dL.

A list of drugs and other interfering substances with HDL cholesterol determination has been reported by Young et al.

**NOTES**

BSM has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

**BIBLIOGRAPHY**

2. US National Cholesterol Education Program of the National Institutes of Health.

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**PRINCIPLE OF THE METHOD**

Directly determination of serum HDLc (high-density lipoprotein cholesterol) levels without the need for any pre-treatment or centrifugation of the sample. The method depends on the properties of a detergent which solubilizes only the HDL to that the HDL-c is released to react with the cholesterol esterase, cholesterol oxidase and chromogens to give colour. The non HDL lipoproteins LDL, VLDL and chylomicrons are inhibited from reacting with the enzymes due to absorption of the detergents on their surfaces.

**CLINICAL SIGNIFICANCE**

HDL particles serve to transport lipoproteins in the blood-stream. HDL is known as ‘good cholesterol’ because high levels are thought to lower the risk of heart disease and coronary artery disease. A low HDL cholesterol level is considered a greater heart disease risk.

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

**REAGENTS**

<table>
<thead>
<tr>
<th></th>
<th>GOOD pH 7.0</th>
<th>GOOD pH 7.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>R 1</td>
<td>Cholesterol oxidase &lt; 1000 U/L</td>
<td>Cholesterol oxidase &lt; 1000 U/L</td>
</tr>
<tr>
<td></td>
<td>Peroxidase &lt;1300 U/L</td>
<td>Peroxidase &lt;1300 U/L</td>
</tr>
<tr>
<td>DSBmL</td>
<td>&lt; 1 mm</td>
<td>&lt; 1 mm</td>
</tr>
<tr>
<td>R 2</td>
<td>Cholesterol esterase &lt; 1500 U/L</td>
<td>Cholesterol esterase &lt; 1500 U/L</td>
</tr>
<tr>
<td></td>
<td>4 - Aminooantipyrine (4-AP) &lt; 1 mm</td>
<td>4 - Aminooantipyrine (4-AP) &lt; 1 mm</td>
</tr>
<tr>
<td></td>
<td>Detergent &lt; 2%</td>
<td>Detergent &lt; 2%</td>
</tr>
<tr>
<td></td>
<td>Ascorbic oxidase &lt; 3000 U/L</td>
<td>Ascorbic oxidase &lt; 3000 U/L</td>
</tr>
</tbody>
</table>

**HDLc/ LDLc CAL**

Calibrator, lyophilized human serum.

**PRECAUTIONS**

HDLc/ LDLc CAL Components from human origin have been tested and found to be negative for the presence of HBsAg, HCV, and antibody to HIV (1/2). However handle cautiously as potentially infectious.

**PREPARATION**

- R 1 and R 2: Are ready to use.
- HDLc/ LDLc CAL: Dissolve the contents with 1 mL of distilled water. Cap vial and mix gently to dissolve contents.

**STORAGE AND STABILITY**

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C and contaminations are prevented during their use. Do not freeze the reagents.

- R 1 and R 2: Once opened is stable 8 weeks at 2-8°C.
- HDLc/ LDLc CAL: Once reconstituted 1 week at 2-8°C or 5 weeks at -20°C.

Do not use reagents over the expiration date.

**SIGNALS OF REAGENT DETERIORATION:**

- Presence of particles and turbidity.

**ADDITIONAL EQUIPMENT**

- Spectrophotometer or colorimeter measuring at 600 nm.
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

**SAMPLES**

Serum or heparinized plasma, free of hemolysis: Anticoagulants containing citrate should not be used. Removed from the blood clot as soon as possible

**STABILITY OF THE SAMPLE:** 7 days at 2-8°C.

**PROCEDURE**

1. **Assay Conditions:**
   - Wavelength: 600 -700 nm
   - Cuvette: 1 cm light path
   - Temperature: 37°C

2. Adjust the instrument to zero with distilled water.

3. Pipette into a cuvette:

   **Sample:**
   - R 1 (µL) 300
   - Calibrator (µL) 300
   - Blank (µL) --

4. Mix and incubate for 5 min at 37°C.

5. Read the absorbance (A) of the samples and calibrator.

6. Add:

   **Sample:**
   - R 2 (µL) 100
   - Calibrator (µL) 100
   - Blank (µL) --

7. Mix and incubate for 5 min at 37°C.

8. Read the absorbance (A) of the samples and calibrator, against the blank.

9. Calculate the increase of the absorbance (A):
   - Delta A
   - A = A

**CALCULATIONS**

- Sample concentration in the sample.

**REFERENCES**