**LDL CHOLESTEROL**

**enzymatic (Colorimetric)**

**Manual Procedure:**

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>Temperature</th>
<th>Correlation</th>
<th>Measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>550 nm (PR)</td>
<td>20-25°C, 32°C</td>
<td>1 cm light path</td>
<td>against blank</td>
</tr>
</tbody>
</table>

**Dispensing:**

1. Mix 100μl of sample with 1000μl of precipitating reagent and keep at room temperature for 10 minutes and then centrifuge at 4000 rpm for 13 minutes. The cholesterol concentration of the supernatant can be determined within 1 hour after centrifugation. Rapeese into test tubes as follows:

2. Standard:
   - 50 μl
   - 50 μl

3. Reagent blank:
   - 50 μl

4. Mix the reagents for 10 minutes at room temperature or for 2 minutes at 37°C, then measure the absorbance of the samples and standard against the reagent blank. The endpoint will be stable for 1 hour.

**Calculation:**

Using a Standard:

1. Concentration of cholesterol in the supernatant = x conc of standard

Using a Racthor:

1. Concentration of cholesterol in the supernatant = Sample Absorbence x Factor as follows:

<table>
<thead>
<tr>
<th>250 μm</th>
<th>520 μm</th>
<th>620 μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>42.62</td>
<td>120.15</td>
<td>1200</td>
</tr>
</tbody>
</table>

**Expected Values:**

<table>
<thead>
<tr>
<th>Category</th>
<th>Lower Limit</th>
<th>Upper Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimal</td>
<td>&lt; 100</td>
<td>&lt; 200</td>
</tr>
<tr>
<td>Normal</td>
<td>100-190</td>
<td>200-290</td>
</tr>
<tr>
<td>Borderline</td>
<td>190-290</td>
<td>290-390</td>
</tr>
<tr>
<td>Somatic</td>
<td>290-390</td>
<td>390-490</td>
</tr>
<tr>
<td>Very high</td>
<td>490-590</td>
<td>590-690</td>
</tr>
<tr>
<td>Extreme</td>
<td>&gt; 690</td>
<td></td>
</tr>
</tbody>
</table>

For Calibration we recommend Fortress enzymatic LDL Cholesterol Standard 1.25mmol/l (480 mg/dl). If this standard is used then the results must be multiplied by 1.1 to allow for the dilution effect of the reagent blank on reagent B. The reagent blank should not be carried out on enzyme standards.

**Linewidth:**

The method will be linear up to 20 mmol/l (774 mg/dl).

Expected Values:

- Optimal: < 100 mg/dl
- Normal: 100-190 mg/dl
- Borderline: 190-290 mg/dl
- Somatic: 290-390 mg/dl
- Very high: 490-590 mg/dl
- Extreme: > 690 mg/dl

Use on Automated Analysers:

- The cholesterol determination is suitable for use on a range of automated analysers. Specific instructions for these applications are available on request from our technical department.
HDL CHOLESTEROL

Precipitant

Kit Contents: BXCD422A

- 2x60ml

Store at 2-8°C

For in-vitro diagnostic use only

Reagent Concentration:

- Phosphotungstic Acid: 55mM/L
- Magnesium Chloride: 25mM/L
- Sodium Chloride: 12.5mM/L (500g/L)

Test Principle:

Low density lipoproteins are precipitated by the addition of phosphotungstic acid in the presence of magnesium ions. The HDL fraction remains in the supernatant and is determined by cholesterol assay.

Reagent Handling and Preparation:

- For micro assays, the reagents are supplied ready to use and will be stable up to the expiry date when stored at room temperature (20-35°C).
- For semimicro assays, prepare the precipitating reagent 4x1 ratio with distilled water (to a final concentration of HDL precipitating reagent, and 1ml distilled water. This is stable up to the expiry date when stored at room temperature (20-35°C).

If 2 additional reagents are required for Cholesterol CRBP/PAF assay: BXCD421A

Manual Procedure:

- Wavelength: 546nm (+/- 1nm)
- Temperature: 30°C or 34°C
- Cuvette: 1mL light path
- Measurement:
  - Against reaction blank

Precipitation Procedure:

- Pipette into test tubes as follows:
  - Mixed on a reciprocal shaker
  - Incubate at 38°C for 3 hours
  - Centrifuge 5000 rpm for 10 minutes at room temperature
  - Remove the clear supernatant in 2 hours and determine the cholesterol content by CRBP/PAF method.

Cholesterol of CRBP/PAF Procedure:

- Only one reagent blank per series. Pipette into test tubes
  - Standard 1: 1ml
  - Standard 2: 1ml

Calculation:

- HDL-Cholesterol = total cholesterol - triglycerides - HDL-Cholesterol

Linearity:

- The method will be linear up to 25mmol/L (774 mg/dL)

Interferences:

- The assay is unaffected by:
  - Uric acid (uric acid < 300mg/dL)
  - Hematocrit factor < 1000 uL/mL

Hemolytic samples (Hb > 500mg/dL) and lipemic samples (Triglycerides < 1200mg/dL)

Lipase samples

With a lipase concentration >10000 U/mL should be diluted 1:10 with 0.9% w/v NaCl before assay. The corresponding result should be multiplied by 10.

An HDL cholesterol is affected by a number of factors such as smoking, exercise, hormones, age and sex. Each laboratory should establish its own reference range and calculate the corresponding reference.

Expected Values (MNCS Guidelines):

<table>
<thead>
<tr>
<th>Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/dL</td>
<td>mg/dL</td>
</tr>
<tr>
<td>&lt;40</td>
<td>&lt;200</td>
</tr>
<tr>
<td>1.04</td>
<td>1.55</td>
</tr>
</tbody>
</table>

Use on Automated Analyzers:

The cholesterol determination is suitable for use on a range of automated analyzers. Specific instructions for these applications are available on request from our Technical Department.

Quality Control:

It is recommended that a laboratory uses normal and abnormal reference sets to verify the performance of any procedure. Results should fall within the specified range for the control.

Fortress Vial/Control Cat No BXCD308/A

If results fall outside the acceptable range appropriate action as determined by the laboratory’s internal quality procedures should be taken.

Health & Safety:

This test is designed for use by suitably qualified laboratory personnel only. Exercise the normal precautions required for the handling of laboratory reagents. Do not ingest the material. Dispose of material according to local guidelines.
TRIGLYCERIDES
GPO-PAP MONOQUICK

**Test Principle:**
Enzymatic colorimetric test.

**Clinical Significance:**
Triglycerides are esters of the fatty acid glycerol with 3 long-chain fatty acids. They are partially synthesized in the liver and partly ingested in foods.

**Reagent Handling and Preparation:**
- Buffer: Store at 2-8°C.
- Standard: Store at 2-8°C.

**Protocol:**
- **Sample:** Collect serum using standard sampling tubes.
- **Reagent:** Dilute Reagent A and Reagent B in a 5:1 ratio.

**Sample Preparation:**
- Centrifuge sample containing precipitate before performing the assay.

**Inter-Assay Precision:**
- **CV%**
  - Control Serum 1: 0.69%
  - Control Serum 2: 1.19%
  - Control Serum 3: 0.70%

**Within-Assay Precision:**
- **CV%**
  - Control Serum 1: 0.79%
  - Control Serum 2: 0.45%
  - Control Serum 3: 0.55%

**Sensitivity:**
- **Mean mg/dL**
  - Control Serum 1: 0.4 mg/dL
  - Control Serum 2: 0.5 mg/dL
  - Control Serum 3: 0.3 mg/dL

**Reagent Concentration:**
- **Standard 1:** 24.2 mg/dL
- **Standard 2:** 39.1 mg/dL

**Calculation:**
- **Reagent Blank:** 100 μL
- **Sample Calculation:**
  - \( \text{Triglyceride concentration (mg/dL)} = \frac{\text{Sample absorbance} \times \text{Sample volume (μL)}}{\text{Reagent blank absorbance} \times 1000 \mu L} \)

**Linearity:**
- Measuring range: 0 to 100 mg/dL (2.14 mmol/L)
- Calculated range: 0 to 200 mg/dL

**Kit Composition:**
- Buffer 1: 24.2 mg/dL
- Buffer 2: 39.1 mg/dL
- Reagent A: 24.2 mg/dL
- Reagent B: 24.2 mg/dL
- Control Serum 1: 0.4 mg/dL
- Control Serum 2: 0.5 mg/dL
- Control Serum 3: 0.3 mg/dL

**Manufacturer:**
Fortress Diagnostics Limited
Unit 2C, Attimo Technology Park, Attimo BT41 1WG (United Kingdom)
T: +44 (0) 394 467676 FAX: +44 (0) 394 669933 www.fortressdiagnostics.com

**Revision:**
REVISED OCT/17