**Reagents**

**Enzymatic Triglyceride Reagent, Cat. No. BD385a/d**

- Contains:
  - 4-aminoantipyrine: 0.5 mmol/L
  - 4-chlorophenol: 4.0 mmol/L
  - ATP: 2.0 mmol/L
  - Lipases: > 4.0 U/mL
  - Glycerol-kinas: > 0.4 U/mL
  - Glycerol-3-fosfato oxidasa: > 1.5 U/mL
  - Peroxidasa: > 2.0 U/mL
  - Solución buffer (pH 6.7 ± 0.1): 50 mmol/L

**Activated Triglyceride Standard, 200 mg/dL, Cat. No. FT7610/s**

Contains glycerol with surfactant to yield 200 mg/dL triglycerides as triolein. Sodium azide 0.1% added as a preservative.

**Precautions:** For In Vitro Diagnostic Use. Reagents and standard contain sodium azide as a preservative. May react with copper or lead plumbing to form explosive metal azides. Upon disposal, flush with large amounts of water to prevent azide build up.

**Reagent Preparation:**

**Cat. No. BD385a/c:** Enzymatic Triglyceride Reagent, should be stored at +4°C.

**Cat. No. BD3862:** Triglyceride standard should be stored at +4°C.

Bring reagent and standard to room temperature before use.

**LiquiColor® Triglycerides Test Procedure No. BD385**

For the Quantitative Enzymatic- Colorimetric Determination of Triglycerides in Serum or Plasma

**Summary and Principle**

Measurement of triglyceride levels, when performed in conjunction with other lipid assays, proves useful in the diagnosis of primary and secondary hyperlipoproteinemia. Triglyceride concentrations are also of interest in following the course of diabetes mellitus, nephrosis, biliary obstruction and various metabolic abnormalities resulting from endocrine disturbances.

1. Glycerol and fatty acids are first formed by lipase action on the triglycerides.
2. Glycerol is then phosphorylated by adenosine-5'-triphosphate (ATP) to produce glycerol-3-phosphate (G-3-P) and adenosine-5'-diphosphate (ADP) in a reaction catalyzed by glycerol kinase (GK):
   
   \[
   \text{Glycerol + ATP} \rightarrow \text{G-3-P} + \text{ADP + H}_2\text{O} \]

3. The G-3-P is oxidized by glyceraldehyde phosphate oxidase (GPO) producing dihydroxyacetone phosphate (DAP) and hydrogen peroxide
   
   \[
   \text{G-3-P + O}_2 \rightarrow \text{DAP + H}_2\text{O} \]

4. Peroxide reacts with a 4-aminoanthipyrine and 4-chlorophenol under the catalytic influence of peroxidase (POD) to form quinonimine
   
   \[
   2\text{H}_2\text{O} + 4\text{-aminoantipyrine} + 4\text{-chloorophenol} \rightarrow \text{quinonimine + HCl + 4H}_2\text{O} \]

Lipid Clearing Factor (LCF): a mixture of specially designed additives (please inquire) is integrated into the triglyceride reagent to help minimize interference due to lipemia.

**Materials Required But Not Provided**

- Spectrophotometer capable of absorbance readings at 500 nm (492-530 nm)
- Accurate pipetting devices
- Heat block or water bath, 37°C (optional)
- Interval Timer, Shaker, cuvets

**Materials Required For Procedure**

- Sodium azide 0.1% added as a preservative.
- Solución buffer (pH 6.7 ± 0.1.)
- Peroxidasa
- Glicerol-3-fosfato oxidasa
- Glicerol-kinasa
- ATP
- 4-aminoantipirina

**Sample Collection and Preparation**

**Sample Stability:** Collect blood using preferably heparin or EDTA anticoagulants. Fluor and owalate should not be used. Separat immediately ke serum ou le plasma. Triglycerides are reportedly stable for at least 10 days at 2-8°C. Do not store samples at 15-25°C as phospholipids may hydrolyze, releasing free glycerol and falsely elevating triglyceride values.

**Interfering Substances:** Blood collecting devices containing glycerol (glycerin) cannot be used, such as those having stoppers so lubricated. Gross hemolysis or high biliurbin values, will produce falsely elevated values. A number of drugs and substances affect the determination of triglycerides.

**Interference from gross icteric and heavily hemolyzed specimens is correctible by use of serum/plasma blank (refer to “Results” section).**

**Automated Analyzer**

**Parameters:**

- **Wavelength:** 500 nm
- **Reaction Type:** Endpoint
- **Reaction Temperature:** 37°C
- **Sample/Reagent Ratio:** 1:100
- **Equilibration Time:** 3 Seconds
- **Read Time:** 4 Seconds
- **Lag Time:** 300 Seconds
- **Blank Absorbance Limit:** 0.500A
- **High Absorbance:** 2,000A
- **Standard:** 200 mg/dL
- **Low Normal:** 30 mg/dL
- **High Normal:** 400 mg/dL
- **Linearity:** 1000 mg/dL

Above parameters should be employed in programmed automated analyzers for Triglycerides. Consult your instrument manual for programming instructions.

**Manual Procedure**

1. Pipet into cuvets the following volumes (mL) and mix well:

<table>
<thead>
<tr>
<th>Activated Reagent Blank (RB)</th>
<th>Standard (S)</th>
<th>Sample (U)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Standard</td>
<td>0.01</td>
<td>-</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>0.01</td>
</tr>
</tbody>
</table>

**Quality Control:** Include control serums with triglyceride values for each run, i.e. our Ser-T-Fy® I, Normal Control Serum, Cat. No. FT7670 and Ser-T-Fy® II, Abnormal Control Serum, Cat. No. FT7680. Triglycerides determined in these materials, by this procedure should fall within the ranges stated for the controls. Two levels of controls should be analyzed with each run.

**Results**

Values are derived by the following equations:

\[ \text{Serum Triglyceride (mg/dL)} = \frac{\text{As}}{\text{Au}} \times 200 \]

where As and Au are the absorbance values of unknown and standard, respectively and 200 the concentration of the standard (mg/dL). When a serum blank is required (icteric or hemolyzed specimen), label another tube SB (Step 1. “Procedure” section). Add 1.0 mL "normal" saline, 0.01 mL serum, mix by inversion, transfer to cuvet and read absorbance (Asb) vs distilled water at 500 nm. Use this value to correct that of the unknown as follows:

\[ \text{Serum Triglyceride (mg/dL)} = \frac{\text{Asb}}{\text{As}} \times 200 \]

NOTE: Samples having triglyceride values greater than 1000 mg/dL are diluted 5-fold (1+4) with normal saline (sodium chloride, 8.5 g/L), the assay repeated and results multiplied by the dilution factor of 5.

**Expected Values**

30 – 150 mg/dL

It is recommended that each laboratory establish its own normal range of expected values, since differences exist between instruments, laboratories, and local populations.

**Performance Characteristics**

**Reproducibility:** A study was performed on a control serum (mean = 57 mg/dL) and a patient pool (mean = 327 mg/dL) which entailed 10 determinations on each for 5 successive days. Coefficients of variation (CV) were within run 1.18% and 0.88% and between runs 1.96% and 0.88%, respectively.

**Correlation:** Determination of triglyceride levels by the procedure described (y) and by the GPO method of Boehringer Mannheim (x) on 57 sera (range 47 to 950 mg/dL) showed a correlation coefficient (r) of .99 and a regression equation of y = 1.029x – 7.46.

**Linearity:** When performed as directed the method is linear from 0 to 1000 mg/dL.

**References**

5. BAN Laboratory data

For any information (technical service call, ordering,...), please contact Interchim 213 av. JF Kennedy, BP 1140 – 03 103 Montlucon (France)

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