**Intended Use**

For the *in vitro* quantitative determination of Triglycerides in serum or plasma. For *in vitro* diagnostic use only.

**Clinical Significance**

Triglycerides determinations are of interest in the diagnosis and treatment of atherosclerosis, poorly controlled diabetes mellitus, nephrosis, liver disease, or other diseases involving lipid metabolism.

**Test Summary**

The triglycerides (GPO) method is based on the enzymatic determination of glycerol using the enzyme glycerol phosphate oxidase (GPO) after hydrolysis by lipoprotein lipase. The principle of this method was described by Fossati\(^1\) who coupled the reaction with the classical Trinder\(^2\) reaction sequence. This single reagent procedure quantitates the total glycerides in serum including the mono and diglycerides, and the free glycerol fractions. This approach is the basis for this method.

**Principle**

\[
\text{Triglycerides} \xrightarrow{\text{Lipase}} \text{Glycerol + Fatty Acids}
\]

\[
\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{Glycerol-1-phosphate + ADP}
\]

\[
\text{Glycerol-1-phosphate + O}_2 \xrightarrow{\text{GPO}} \text{DAP} + \text{H}_2\text{O}_2
\]

\[
\text{H}_2\text{O}_2 + 4\text{-AA + 4-Chlorophenol} \xrightarrow{\text{POD}} \text{Quinoneimine Dye + HCL + 2H}_2\text{O}
\]

Serum triglycerides are hydrolyzed to glycerol and free fatty acids by lipase. In the presence of ATP and glycerol kinase (GK), the glycerol is converted to glycerol-1-phosphate. The glycerol-1-phosphate is then oxidized by glycerol phosphate oxidase (GPO) to yield hydrogen peroxide. The condensation of hydrogen peroxide with 4-chlorophenol and 4-aminophenazone (4CAA) in the presence of peroxidase (POD) produces a red colored quinoneimine dye which absorbs at, or near 505nm. The intensity of the colored complex formed is directly proportional to the triglyceride concentration of the sample.

**Reagent Composition**

- 4-Chlorophenol 3.5mM, ATP >0.5mM, Magnesium salt 10 mM, 4-Aminophenazone 0.3mM, Glycerol Kinase (microbial) >250 U/L, Glycerol Phosphate Oxidase (microbial) >4500U/L, Peroxidase (horseradish) > 2000 U/L, Lipase (microbial) >200,000 U/L, buffer (pH 7.3 ± 0.1), surfactants, stabilizers, and preservatives, including sodium azide (0.01%).

**Reagent Preparation**

The reagent is ready to use.

**Reagent Storage and Stability**

Store the reagent at 2-8°C. The reagent is stable until the expiration date appearing on the label when stored as directed. Protect from direct light. Avoid microbial contamination.

Do not use the reagent if:

1. The initial absorbance of the reagent is greater than 0.350 when measured at 505nm against water in a cuvette with a one centimeter path length.
2. The reagent is turbid or displays evidence of bacterial contamination.

**Precautions**

1. This reagent set is intended for *in vitro* diagnostic use only.

**Materials Provided**

- Triglycerides (GPO) reagent

**Materials Required but not Provided**

1. Beckman Coulter AU™ analyzer
2. Calibrator
3. Controls
4. Instrument and application manuals

**Procedure (Beckman Coulter AU400 application)**

**SPECIFIC TEST PARAMETERS**

<table>
<thead>
<tr>
<th>TEST NUMBER:</th>
<th>TEST NAME: Trig V</th>
<th>TYPE: Serum V</th>
<th>OPERATIONAL: Yes V</th>
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<td>PRE-DILUTION RATE: 1</td>
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<td>REAGENTS: R1 VOLUME: 200</td>
<td>DIL. VOL: 0</td>
<td>MIN. OD</td>
<td>MAX. OD</td>
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<td>R2 VOLUME: 0</td>
<td>DIL. VOL: 0</td>
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<td>H</td>
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<td>660 V</td>
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<tr>
<td>REAGENT OD LIMIT:</td>
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<tr>
<td>NO LA G TIME:</td>
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<td>L:</td>
<td>H:</td>
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</tr>
<tr>
<td>LINEARITY:</td>
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<td>A: 1.000</td>
<td>B: 0.000</td>
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</table>

1. The reagent contains sodium azide (0.01%) as a preservative. Do not ingest. Avoid skin and eye contact. Sodium azide may react with copper or lead plumbing to form explosive metal azides. Upon disposal flush with large amounts of water.
2. All specimens and controls should be handled as potentially infectious. Use safe laboratory procedures. (NCCLS M29-T2)^3^

**Specimen Collection and Storage**

1. Fresh, clear, unhemolyzed serum is the specimen of choice. The specimen should be collected following the guidelines of NCCLS document H4-A3.\(^4\)
2. The specimen should be collected following a 12 hour fast, and separated from the clot as soon as possible. Avoid anticoagulants containing fluoride or oxalate.
3. Serum or plasma may be stored for one week at 2-8°C or for three months at -20°C.\(^5\)
4. Frozen samples should be thawed at room temperature and mixed completely before analysis. Thawed samples should not be refrozen.

**Interferences**

1. A number of drugs and substances affect the determination of triglycerides.\(^6,7\) Young, et al\(^8\) have published a comprehensive list of these substances.
2. The method is not influenced by hemoglobin values up to 100mg/dl(<5%). Elevated levels of Bilirubin can interfere with this assay, causing decreased recovery of triglyceride concentrations.
3. Detergents can interfere with the action of lipase. Care should be taken to avoid contamination of laboratory equipment with detergents.

**Materials Provided**

- Triglycerides (GPO) reagent

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Triglycerides (GPO) Reagent Set

Triglycerides = 157 mg/dl

Note: To convert the results into SI units (mmol/L), multiply the result (mg/dl) by 0.0113.

Expected Values

44-148 mg/dl (0.50-1.67 mmol/L)

Due to a wide range of conditions (dietary, geographical, age, etc.) believed to affect normal ranges, it is recommended that each laboratory establish its own reference range.

Performance

1. Linearity: 1000 mg/dl (11.3 mmol/L). Samples that exceed 1000 mg/dl should be diluted with an equal volume of saline and re-assayed. Multiply the result by two.

2. Comparison: A comparison study performed between the Beckman Coulter AU400 and Roche Hitachi 717 using this method resulted in a correlation coefficient of r = 1.000 and a linear regression equation of: y = 1.045x + 2.05. (n=37, range 48–701 mg/dl)

3. Precision:
   - Within - day precision study was performed using three levels of material. Between - day precision study was performed using two levels of control material assayed over a 20 day period with 2 runs per day and 2 replicates per run.

   Within Day (N=20)          Day to Day
   Mean  S.D.  C.V.%  Mean  S.D.  C.V.%
   12    0.5    4.2    100  3.7    3.7
   105   1.6    1.5    203  5.5    2.7
   270   4.7    1.7

Precision and Linearity studies were performed following modifications of CLSI Protocols EP-5 and EP-6 using a Beckman AU400 analyzer.

4. Sensitivity: The sensitivity for this product was investigated by reading the change in absorbance at 520/660 nm for a saline sample, and serum samples with known concentrations. Ten replicates were performed. The results of this investigation indicated that, on the analyzer used, this product showed little or no drift on a zero sample. Under the reaction conditions described, 1mg/dl of triglycerides gives an absorbance of 0.001.

References


Calculation

Triglycerides results are expressed as mg/dl or mmol/L.

Triglycerides = Abs Std x Conc. Std

Example:
Abs Std = 0.310
Conc. Std = 200 mg/dl
Triglycerides = 0.243 x 200 mg/dl

0.310