

# Manual Procedure

**Cat. No. 12851** R 2 x 50 ml  
For 100 tests

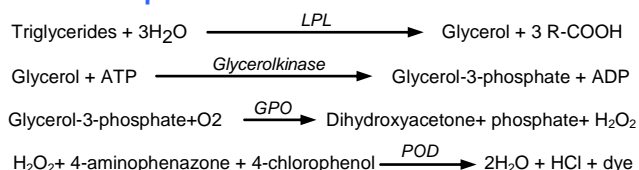
**Cat. No. 12852** R 6 x 50 ml  
For 300 tests

## Triglycerides GPO/PAP

Enzymatic colorimetric method

### Liquid mono Reagents

#### Test Principle



**dye = 4-(p-benzoquinone-monoimino)-phenazone**

Serum triglycerides are hydrolyzed to glycerol and free fatty acids by lipoprotein lipase. In the presence of ATP and glycerol kinase (GK), the glycerol is converted to glycerol-3-phosphate, which then is oxidized by glycerol phosphate oxidase (GPO) to yield hydrogen peroxide. The oxidative condensation of 4-Chlorophenol and (4-AAP) 4-aminophenazone in the presence of Peroxidase (POD) and hydrogen peroxide produces a rose colored dye which is measured at 550 nm. The intensity of the colour formed is directly proportional to the triglycerides concentration in the sample.

#### Concentration of the test

| Reagent R   |       |        |
|---|-------|--------|
| PIPES [Piperazine-1,4-bis (2-ethane-sulfonic acid)]       | 50.0  | mmol/L |
| EDTA  | 0.13  | mmol/L |
| ATP (Adenosine-tri-phosphate)                             | 1.65  | mmol/L |
| Magnesium ions  | 0.5   | mmol/L |
| 4-Aminophenazone  | 0.6   | mmol/L |
| 4-Chlorophenol  | 1.55  | mmol/L |
| GPO (Glycerophosphate-Oxidase)                            | ≥ 2.5 | KU/L   |
| Glycerolkinase  | ≥ 1.0 | KU/L   |
| POD (Peroxidase)  | ≥ 5.0 | KU/L   |
| LPL (Lipoprotein lipase)                                  | ≥ 2.0 | KU/L   |
| Detergent, Stabilizer, preservative.                      |       |        |
| <b>Standard</b> : The Concentration as indicated on vial. |       |        |

#### Stability and preparation of working reagent

**Reagent R:** liquid mono reagent, ready to use.

The reagent is stable up to expiry date given on the label when stored at +2 → +8 °C. Stability after opening the bottle: 2 months at +2 → +8 °C.

**Note:** Don't use if the initial absorbance of the reagent is greater than 0.350 when measured at 550 nm against water.

#### Specimen collection and handling

1. Non-hemolyzed serum, heparinized or EDTA plasma is recommended.
2. Avoid anticoagulants containing fluoride or oxalate.
3. The serum should be collected following a 12 hour fasting, and separated from the clot as soon as possible.
4. Serum or plasma may be stored for 1 week at 2 - 8 °C and for 3 months at - 20 °C.
5. Frozen samples should be thawed at room temperature and mixed completely before analysis. Thawed samples should not be refrozen.

#### Calibrator / Standard

MediCal U Cat. No. 15011  
Triglycerides STD. Cat. No.16191

#### Quality control

Meditrol N Cat. No. 15171  
Meditrol P Cat. No. 15181

#### Procedure

|                   |                       |
|-------------------|-----------------------|
| Wavelength        | Hg 546 (500 - 560 nm) |
| Spectrophotometer | 505 nm                |
| Cuvette           | 1 cm light path       |
| Temperature       | 37 °C / 20 - 25 °C    |
| Measurement       | against reagent blank |
| Reaction          | end point             |

#### Assay

|  | Blank   | Calibrator / Standard | Sample  |
|--|---------|-----------------------|---------|
| Distilled water  | 10 µl   | --                    | --      |
| Calibrator / Standard  | --      | 10 µl                 | --      |
| Sample   | --      | --                    | 10 µl   |
| Working Reagent  | 1000 µl | 1000 µl               | 1000 µl |
| Mix, incubate for 5 min. at 37 °C or 10 min. at 20 - 25°C. Read the absorbance (A). The final color is stable for at least 30 min. |         |                       |         |

#### Calculation

$$\text{Conc. Triglycerides (mg/dl)} = \frac{A_{\text{Sample}}}{A_{\text{Cal./STD.}}} \times \text{Conc. Cal./STD. (mg/dl)}$$

$$\text{mmol} \xrightleftharpoons[0.0114 \times]{\times 87.5} \text{mg/dl}$$

#### Linearity

Up to 1000 mg/dl (11.43 mmol/L).

If the result exceeds 1000 mg/dl, repeat the test using diluted serum (1+4) with sodium chloride solution (0.9 %) and multiply the result by 5.

#### Interferences

1. A number of drugs and substances affect the determination of triglycerides. Young, et al, have published a comprehensive list of these substances.<sup>4</sup>
2. The method is not influenced by bilirubin levels up to 12 mg/dl (< 5 %).
3. Detergents can interfere with the action of lipase. Care should be taken to avoid contamination of laboratory equipment with detergents.
4. Ascorbic acid: No significant interference up to 100 mg/dl.
5. Hemolysis interferes with the test.

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### Precaution

The reagent contains sodium azide (0.1 %) as a preservative. Don't 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.

### Reference range

|        |       |       |
|--------|-------|-------|
| Adults | < 200 | mg/dl |
|--------|-------|-------|

### References

1. Trinder, C. *Klin. Chem. klin. Biochem.* 8 (1970) 658.
2. Weibhaar, D. Grossau, E. und All., *Med. Welt* 26 (1975) 387-390.
3. Kubler, W., *Symp. der Deutschen Gesellschaft für Lab. Med. Mainz* (1973).
4. Young, D.S., *Effects of Drugs on Clinical Laboratory Tests*, fifth edition 2000, AACC Press, Washington, D.C.