

Intended Use

The reagents are used for the quantitative determination of Triglycerides in human sera or plasma. For in-vitro diagnostic use only.

Introduction

Triglycerides are synthesized in intestinal epithelial cells by the esterification of glycerol and fatty acid. Once formed, they combine with other lipids and lipoproteins in complex assemblages known as chylomicrons, which are released from the intestinal epithelium into the circulatory system. Elevated serum triglyceride levels are associated with increased risk of coronary artery disease, pancreatitis, glycogen storage disease, biliary cirrhosis and numerous genetic lipoprotein disorder.

Method

Glycerol Phosphate Oxidase-Peroxidase method, End Point.

Principle

Serum triglycerides are hydrolyzed to glycerol and free fatty acids by lipase. In the presence of ATP and Glycerol Kinase (GK), the glycerol is phosphorylated to glycerol-3-phosphate (Glycerol-3-P), which is then oxidized by glycerol phosphate oxidase (GPO) to yield dihydrogen acetone phosphate and hydrogen peroxide (H₂O₂). The hydrogen peroxide oxidizes a chromogen under the influence of enzyme peroxidase (POD) to form a red quinoneimine dye. The intensity of red colored complex is measured at 505 nm.



Reagent Composition

Reagent 1:

| | |
|----------------------------|---------------|
| Lipoprotein lipase | > 2000U/l |
| 4-Amino Antipyrine | 0.246 mmole/l |
| Peroxidase | > 500U/l |
| Glycerol kinase | > 300 U/l |
| Glycerol Phosphate Oxidase | > 1000U/l |

Reagent 2:

| | |
|-----------------------|-----------|
| Triglyceride Standard | 200 mg/dl |
|-----------------------|-----------|

Precautions

Following precautions should be taken:

- Avoid ingestion, do not pipette by mouth.
- Avoid contact with skin and eyes. If spilled, thoroughly wash affected area with water.
- Flush with plenty of water while disposing.

Reagent Storage and Stability

Unopened Reagents 1 are stable till expiry mentioned on the label when stored at 2–8°C.

Standard Reagent 2 is stable till expiry as mentioned on the label when stored at 2-8°C.

Note: On request, Reagent 3 (500 mg/dl) & Reagent 4 (1000 mg/dl) can be provided for linearity check with Reagent 2 (Triglyceride Standard: 200 mg/dl)

Reagent Preparation

Reagents are ready for use.

Reagent Deterioration

Reagents should be clear. Turbidity and/or precipitation may be because of reagent deterioration.

Sample Collection and Storage

Unhaemolysed serum or plasma can be used for the testing. Anti-coagulants like EDTA and heparin can be used. It is recommended to use freshly collected samples for assay. Serum samples can be stored for 3 days at 2-8°C.

General Assay Parameters

| Mode | End Point |
|------------------------------|-----------|
| Wavelength (nm) | 505 |
| Wavelength Range Usable (nm) | 500-550 |
| Blank with | Reagent |
| Sample Volume (µl) | 5/10 |
| Reagent 1(µl) | 500/1000 |
| Incubation Time (min.) | 10 |
| Incubation Temperature (°C) | 37 |
| Normal Low (mg/dl) | 25 |
| Normal High (mg/dl) | 170 |
| Linearity (mg/dl) | Upto 1000 |
| Standard Conc. (mg/dl) | 200 |
| Units | mg/dl |

PROCEDURE

One reagent blank and one standard are sufficient for each assay series.

Pipette into test tubes:

| Particulars | Blank | Standard | Sample |
|-------------|---------|----------|---------|
| Reagent 1 | 1000 µl | 1000 µl | 1000 µl |
| Dist. Water | 10 µl | - | - |
| Reagent 2 | - | 10 µl | - |
| Sample | - | - | 10 µl |

Mix well & incubate for 20 min. at 20-25°C or 10 min. at 37°C. Measure the absorbance of standard (A std) and sample (A sample) against reagent blank at 505 nm.

Calculation

Triglyceride concentration in the sample can be calculated using the following formula:

$$\text{Triglyceride (mg/dl)} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times \text{Conc. of Std. (mg/dl)}$$

Example: If the absorbance of sample is 0.072 and the absorbance of standard is 0.178. The calculation shall be:

$$\frac{0.072}{0.178} \times 200 = 80.8 \text{ mg/dl}$$

If the triglyceride concentration exceeds 1000mg/dl, dilute the sample with normal saline and repeat the assay. The reportable results in this case shall be calculated by multiplying the results obtained with dilution factor.

Reference value

Normal upto 170 mg/dl

Reference range varies from population to population; therefore, each laboratory should establish its own normal range.

Limitations

- 1.The reagent and sample volumes can be altered proportionately so that the sample: reagent, ratio remains same.
2. Gross hemolysis and lipemia may result in false elevated results.

Quality Control

The patient results obtained for each batch can be validated by using normal and abnormal control sera with assayed values for triglyceride.

Performance

Linearity Limit: 1000mg/dl

Precision:

Within run

| Control | Control 1 | Control 2 |
|----------------|-----------|-----------|
| No. of samples | 20 | 20 |
| Mean (mg/dl) | 83.74 | 157.40 |
| S.D. | 1.45 | 0.62 |
| C.V. % | 1.73 | 0.39 |

Between run

| Control | Control 1 | Control 2 |
|----------------|-----------|-----------|
| No. of samples | 60 | 60 |
| Mean (mg/dl) | 4.05 | 10.98 |
| S.D. | 0.07 | 0.06 |
| C.V. % | 1.70 | 0.57 |

References

- 1."Your Triglyceride Level". What Your Cholesterol Levels Mean.American Heart Association http://www.americanheart.org/presenter.jhtml?identifier=183#_triglyceride. Retrieved on 2009-05-22.
- 2."Fish and Omega-3 Fatty Acids". American Heart Association. <http://www.americanheart.org/presenter.jhtml?identifier=4632>Retrieved on 2009-05-22.
- 3.Balch, Phyllis A. Prescription for nutritional healing. 4th ed.New York: Avery, 2006. p. 54 Carnitine
- 4.<http://www.webmd.com/cholesterol-management/tc/high-triglycerides-overview>
- 5.Burtis, C., and Ashwood, E.R., Editors, Tietz Textbook of Clinical Chemistry, 3rd edition, W.B. Saunders Co., Toronto, 1999+triglyceride <http://www.genzymediagnosics.com/resources/PI/Triglyceride-SL.pdf>

Pack Presentation

| Product Code /Catalogue No. | Pack Size* | Reagent 1 | Reagent 2 |
|-----------------------------|------------|-----------|-----------|
| KGTRG103.4.1 | 2x50ml | 2x50ml | 1x3ml |
| KGTRG103.4.2 | 5x50ml | 5x50ml | 1x3ml |
| KGTRG103.4.3 | 5x100ml | 5x100ml | 1x3ml |

* Pack size may vary on market demand.

Revision No: (Ver: KGTRG103.4/1)

Date of Issue: 1st April 2010

Symbols

Following symbols are used in the labeling of KEE GAD kits:

| | | | |
|--|----------------------|--|---------------------|
| | Catalogue No. | | Batch No. |
| | CE Mark | | Read instructions |
| | In Vitro Diagnostics | | Storage temperature |
| | Expiry Date | | Content |
| | Product Name | | Manufactured By |



Manufactured by:

KEE GAD Biogen Pvt. Ltd.

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