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TRIGLYCERIDE-GPO REAGENT SET

INTENDED USE
For the quantitative determination of triglycerides in serum or plasma.

INTRODUCTION
Triglycerides are esters of fatty acids and are hydrolyzed to glycerol and free fatty acids. Triglyceride determinations when performed in conjunction with other lipid assays are useful in the diagnosis of primary and secondary hyperlipoproteinemia. They are also of interest in following the course of diabetes mellitus, nephrosis, biliary obstruction, and various metabolic abnormalities due to endocrine disturbances.

Standard methods for the measurement of triglyceride concentrations involved either enzymatic or alkaline hydrolysis to liberate glycerol. This formulation makes use of the enzymatic hydrolysis and quantification since it is specific and not subject to interference by phospholipids.[1]

PRINCIPLE
The enzymatic reaction sequence employed in the assay of Triglycerides is as follow:

$$\text{Triglycerides} \xrightarrow{\text{Lipase}} \text{Glycerol} + \text{Fatty Acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol Kinase}} \text{Glycerol-l-phosphate} + \text{ADP}$$

$$\text{Glycerol- 1-Phosphate} + O_2 \xrightarrow[\text{Oxidase}]{\text{GIP}} \text{DAP} + H_2O_2$$

$$H_2O_2 + 4\ \text{AAP} + \text{DHBS} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine Dye} + 2H_2O$$

The present procedure involves hydrolysis of triglycerides by lipase. The glycerol concentration is then determined by enzymatic assay coupled with Trinder reaction that terminates in the formation of a quinoneimine dye. The amount of the dye formed, determined by its absorption at 520 nm, is directly proportional to the concentration of triglycerides in the samples.[2, 3]

REAGENT COMPOSITION
When reconstituted as directed, the reagent for Triglycerides contains the following:
1. Triglyceride reagent (Concentrations refer to the reconstituted reagent): ATP 3.3mM, Magnesium salt 3.0mM, 4-Aminoantipyrine 0.7mM, 3,5-Dichloro-2-hydroxybenzene sulfonate 0.8mM, Glycerol-l-Phosphate Oxidase 7000 U/L Sodium azide 0.01%, Lipase 200,000 U/L, Glycerol kinase 1000 U/L, Peroxidase 10,000 U/L, Buffer 50mM, pH 7.6 ± 0.2.
2. Triglyceride standard: Contains glycerol with surfactant to yield 200 mg/dl triglycerides as triolein. Sodium azide 0.1% is added as a preservative.

WARNINGS AND PRECAUTIONS
1. For *in vitro* diagnostic use.
2. Avoid ingestion of reagent, as toxicity has not yet been determined.
3. Specimens should be considered infectious and handled appropriately.
4. Reagent and standard contain sodium azide as a preservative. This may react with copper or lead plumbing to form explosive metal azides. Upon disposal, flush with large amount of water to prevent azide build up.

STORAGE AND STABILITY
Both the triglyceride reagent and standard must be stored at 2 - 8°C prior to reconstitution. The reagent may be used until the expiration date indicated on the package label. After reconstitution the reagent is stable for at least thirty (30) days when stored at 2 - 8° C and seven (7) days at room temperature. The reagent must be protected from light.

REAGENT DETERIORATION
The reagent should be discarded if:
1. The dry powder appears moist and has a dark discoloration.
2. The reagent fails to meet linearity claims or fails to recover stated values.
   Note: A yellow or pink coloration is normal.
3. The reconstituted reagent has an absorbance of 0.5 or greater against water at 520 nm.

SPECIMEN COLLECTION
1. Fresh, clear, non-hemolyzed serum from fasting patients is recommended.
2. Triglycerides in serum appear stable for three (3) days when stored at 2 - 8°C.[4]
3. Prolonged storage of the samples at room temperature is not recommended since other glycerol containing compounds may hydrolyze, releasing free glycerol.
4. Blood collection devices lubricated with glycerin (glycerol) should not be used.

INTERFERING SUBSTANCES
Glycerol in rubber stoppers or in contaminated glassware will elevate triglyceride levels. Lipemic or grossly icteric samples will cause falsely elevated results consequently a patient blank should be run. Samples with gross hemolysis or high bilirubin values will produce falsely elevated triglyceride values. A number of drugs and substances affect the measurement of triglyceride.[5]

MATERIALS REQUIRED BUT NOT PROVIDED
1. Spectrophotometer capable of measuring absorbances at 520nm.
2. Cuvettes
3. Pipettes capable of accurately measuring required volumes (1.0 ml, 2.0 ml, 0.01 ml, 0.02 ml)
4. Constant temperature incubator set at 37° C
5. Timer
6. Distilled water

AUTOMATED PROCEDURE
Consult our appropriate instrument application instructions.
Note: Certain instruments require different reconstitution volumes than those stated on the vial label. Refer to appropriate application sheets.

MANUAL PROCEDURE
1. Reconstitute the triglyceride reagent according to vial label instructions.
2. Label tubes: blank, standard, control, unknown, etc.
3. Pipette l.0 ml of reagent into all tubes.
4. Place all tubes in a 37° C heating block for at least 4 minutes.
5. Add 0.01 ml (10 µl) of sample to respective tubes and mix.
6. Incubate all tubes for five (5) minutes at 37° C.

7. Zero spectrophotometer at 520 nm with reagent blank (Wavelength range: 500 – 550 nm).
8. Read and record absorbances of all tubes.

Note: Final color is stable for sixty (60) minutes at room temperature.

* *TC MULTI-PURPOSE CALIBRATOR MAY BE USED TO REPLACE STANDARD.*

ALTERNATE VOLUMES

For spectrophotometers requiring more than 1.0 ml of reagent add 0.02 ml (20 µl) of sample to l.0 ml of reagent. After 10 minutes of incubation at 37°C add 2.0 ml of distilled water to all tubes, invert to mix, and read immediately at 520 nm.

LIMITATIONS

The reagent is linear to 1000 mg/dl Triglycerides. Samples with values above 1000 mg/dl should be diluted with water, reassayed and the results multiplied by the dilution factor.

CALCULATIONS

A = Absorbance

$$\frac{A\ (unknown)}{A\ (standard)} \times \text{Concentration of standard (mg/dl)} = \text{Triglyceride value of unknown (mg/dl)}$$

Example: $\frac{0.24}{0.31} \times 200 = 154.8$ mg/dl

NOTE: To obtain the results in SI units (mmol/L) multiply the result in mg/dl by 10 to convert dl to liter and divide the value by 885, the molecular weight of triglycerides as triolein.

Example:

$$154.8 \text{ mg/dl} \times \frac{10}{885} = 1.75 \text{ mmol/L}$$

QUALITY CONTROL

It is recommended that controls be included in each set of assays. Commercially available control material with established triglyceride values may be used for quality control. The assigned value of the control material must be confirmed by the chosen application. Failure to obtain the proper range of values in the assay of control material may indicate either reagent deterioration, instrument malfunction, or procedural errors.

EXPECTED VALUES

36 – 165 mg/dl[6]

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

PERFORMANCE CHARACTERISTICS

1. *Linearity:* 1000 mg/dl
2. *Sensitivity:* Based on an instrument resolution of A = 0.001, this procedure has a sensitivity of 0.64 mg/dl.
3. *Comparison:* A comparison of this procedure with our INT procedure was performed on 22 human sera. A correlation coefficient of 0.98 and a linear equation of y = l.0 x + 2.03 were obtained throughout a range of values up to 289 mg/dl.
4. *Precision studies:*
   Within Run: Two commercial control sera were assayed 15 times and the following run precision was obtained (N= 15).

Mean (mg/dl)	S.D.	C.V.
84	4	4%
200	8	4%

Run-to-Run: Two commercial sera were assayed for period of 30 days and the following day-to-day precision was obtained (N= 14).

Mean (mg/dl)	S.D.	C.V.
82	3	3%
199	7	3%

5. *Specificity*: This procedure measures total triglycerides found in the serum, as well as free glycerol present in the sample. In fresh serum, the concentration of free glycerol does not generally exceed 1.0 mg/dl (9.6 mg/dl triglyceride).[7]

REFERENCES

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