UDI
المصنع المتحد للكواشف الطبية
ص. ب ٩٤٦٦ - الدمام ٣١٤١٣ - المملكة العربية السعودية
تلفون : ٨١٢ ٢٠٠٤ - ٨١٢ ١٢٣٣ (٠٣) - فاكس : ٨١٢ ١٨٠٤ - ترخيص رقم ١٩٨٠ / ص
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TRIGLYCERIDES (GPO) REAGENT SET

REF 059L

FOR IN VITRO DIAGNOSTIC USE

INTENDED USE

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

DIAGNOSTIC SIGNIFICANCE

Elevated levels of both cholesterol and triglycerides in plasma have been identified as risk factors related to atherosclerotic disease. The hyperlipidemias can be inherited traits or they can be secondary to a variety of disorders or diseases including diabetes mellitus, nephrosis, biliary obstruction and metabolic disorders associated with endocrine disturbances. The Levels of cholesterol and triglycerides in plasma can vary independently. Therefore, an evaluation of hyperlipidemias should include the determinations for both of these lipids[1].

RANGE OF EXPECTED VALUES IN SERUM[2]

Normal distributions vary with age and according to Fredrickson [2], the following concentrations if exceeded, clearly indicate hyperlipidemia.

0 - 29 years ..... 10 - 140 mg/dL
30 - 39 years..... 10 - 150 mg/dL
40 - 49 years..... 10 - 160 mg/dL
50 - 59 years ..... 10 - 190 mg/dL

As with all "normal" values these values should be checked in each laboratory.

METHOD PRINCIPLE

Standard methods for the measurement of triglycerides concentration have involved either an enzymatic or an 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[3].

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

Triglycerides + H_2O —Lipase—> Glycerol + Fatty Acids

Glycerol + ATP —Glycerol Kinase—> Glycerol-3-Phosphate + ADP

Glycerol-3-Phosphate + O_2 —GPO—> DAP + H_2O_2

H_2O_2 + 4AAP + 4 chlorophenol —Peroxidase—> 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 505± nm is directly proportional to the concentration of triglycerides present in the sample [4, 5].

REAGENTS COMPOSITION

R1. TRIGLYCERIDES BUFFER REAGENT:

Pipes Buffer 40 mmol/L, pH-7.5
4- Chlorophenol 5.0 mmol/L, Magnesium-ions 5.0 mmol/L,

R1a. TRIGLYCERIDES ENZYME REAGENT :

ATP 3.3 mM, 4-Aminoantipyrine 0.7 mM, Glycero-3-Phosphate Oxidase 7000 U/L Sodium Azide 0.01%, Lipase 200,000 U/L, Glycerol Kinase 100 U/L and peroxidase 3,000 U/L.

TRIGLYCERIDES STANDARD (200 mg/dL as Triolein) :

2.2584 mmol/L of Glycerol with Surfactant. Sodium azide 0.01% Added as a preservative.

REAGENT PREPARATION

Reconstitute Triglycerides Enzyme Reagent with the amount of Triglycerides Buffer Specified on the label.

The Reconstituted reagent is stable for 2 days at room temperature (or) 14 days when stored at 2-8 ° C and protected from light.

WARNING AND PRECAUTIONS

1. For in vitro diagnostics use.
2. Avoid ingestion of reagent as toxicity has not yet been determined
3. Specimens should be considered as infectious and handled appropriately.
4. Reagents 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 amounts of water to prevent azide build up.

STORAGE AND STABILITY

Store all reagents included in this reagent set at 2-8 °C prior to working reagent preparation. All reagents are stable upto expiration date indicated on the bottle label.

REAGENT DETERIORATION

The reagent should be discarded if:

1. the reagent fails to meet linearity claims or fails to recover stated values.
   Note : A slight violet coloration is normal.
2. the Reconstituted reagent has an absorbance of over 0.200 at 510 nm.

MATERIALS PROVIDED

Tg-Buffer Reagent (R1), Tg-Enzyme Reagent (R1a) and Tg Standard (200 mg/dL).

MATERIALS REQUIRED BUT NOT PROVIDED

Spectrophotometer, Cuvettes, Pipettes, Constant temperature incubator set at 37 °C, Timer and Distilled water.

SPECIMEN

SERUM

1. Fresh, non-hemolyzed serum from fasting patients is recommended.
2. Triglycerides in serum appears stable for three (3) days when stored at 2-8 °C[6].
3. Prolonged storage of the samples at room temperature is not recommended since other glycerol containing compounds may hydrolyze, releasing free glycerol with an apparent increase in total triglycerides content.
4. Blood collection devices lubricated with glycerin (glycerol) should not be used.

INTERFERING SUBSTANCES

Glycerol in rubber stoppers or in contaminated glassware will elevate triglycerides level. 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 triglycerides values. A number of drugs and substances affect the measurement of triglycerides [7].

QC 011/ 059L Issue 05, Issued on May , 2005 EDMA CODE 11.02.01.31.00.

PROCEDURE (AUTOMATED)

Refer the appropriate application manual available from us

Note: Certain instruments require different working reagent volume than those stated on the vial label. Refer the appropriate instrument application sheets.

PROCEDURE (MANUAL)

Pipette into clean dry test tubes

	BLANK	STANDARD	TEST
Reconstituted Reagent	1.0 ml	1.0 ml	1.0 ml
Pre-warm at 37 °C and add:			
Standard	--	0.01 ml	--
Sample	--	--	0.01 ml
Mix and incubate at 37 °C for 10 minutes, Read the absorbance of standard and sample at 505 ± 5 nm against blank.			

Note: Final color is stable for 30 minutes at room temperature.

ALTERNATE METHOD

For spectrophotometers requiring more than 1.0 ml of reagent, add 0.025 ml (25 µl) of sample to 2.5 ml of reagent. After 10 minutes of incubation at 37 °C read at 505 ± 5 nm.

PROCEDURE LIMITATIONS

This reagent is linear up to 1000 mg/dL Triglycerides. Samples with values above 1000 mg/dL should be diluted with water, re-assayed and the results to be multiplied by 2.

CALCULATIONS

A=Absorbance

$$\frac{A\ (TEST)}{A\ (STANDARD)} \times \text{CONC. OF STD. (mg/dL)} = \text{CONCENTRATION IN TEST (mg/dL)}$$

EXAMPLE: $\frac{0.17}{0.22} \times 200\ mg/dL = 154.5 mg/dL$

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

EXAMPLE: $154.8\ mg/dL \times \frac{10}{885} = 1.75\ mmol/L$

PERFORMANCE CHARACTERISTICS

1. Linearity: 1000 mg/dL
2. Sensitivity : Based on an instrument resolution of A=0.001, this procedure has a sensitivity of 0.6 mg/dL.

COMPARISON: UDI reagents tested by this method (y) was compared with similar UDI reagent for other systems (x), which in turn is matching with CAPS survey results. The reagent was also compared with another commercial reagent. The systematic difference between the results were within CLIA specified limits, N = 36

Correlation Coefficient 0.973

Regression Equation y = 1.2x - 0.59

PRECISION:

	Mean mg/dL	SD	CV%
Within run	101.1	1.96	1.94
Run to run	178	6.31	3.54

5. Specificity: This procedure measure total triglycerides found in the serum, the concentration of free glycerol does not generally exceed 1.0 mg/dL (9.6 mg/dL triglycerides)[8].

QUALITY CONTROL

For accuracy and precision check, we recommend the use of normal and abnormal UDI controls based on human serum.

ORDERING INFORMATION:

UDITROL 'N' (Normal Serum Control) REF # 070N-010 2x5 ml
UDITROL 'A' (Abnormal Serum Control) REF #070A-010 2x5 ml

REFERENCES

1. Tietz, NW, Fundamentals of Clincal Chemistry, Philadelphia, WB Saunders, Second Edition, Page 496.
2. Frederickson, T.F. Mod. Con. of Cardiovasc. Dis. Vol XLI, July 1972, p31.
3. Searcy, R.L. Diagnostic Biochemistry, McGraw-Hill, New York (1969).
4. Fossati, P., Principle, L.: Clin. Chem. 28:2077 (1982).
5. McGowan, M.W. et al. : Clin. Chem. 29:538 (1983).
6. Wybenga, R.D. and Inkpen, J.A. Clinical Chemistry: Principles and Techniques. Harper and Row, Hagerstown, MD 1460 (1974).
7. Young, DS et al, Clin Chem, 21:1D (1975).
8. Tiffany, T.O. et al. Clin. Chem 20:476 (1974).

PRODUCT AVAILABILITY

TRIGLYCERIDES (GPO) LIQUID REAGENT SET (COLOR/ENDPOINT)

REF # 059L-150	**10 x 15 ml**
REF # 059L-100	**2 x 50 ml**
REF # 059L-050	**1 x 50 ml**

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