Intended Use
For **IN VITRO quantitative** determination of Triglycerides in serum.

Clinical Significance
Determination of Triglycerides are primarily used for diagnosing atherosclerosis and heart disease, diabetes mellitus, nephritis, biliary obstruction and metabolic disorders associated with endocrine disturbances, as well as for monitoring the causes and treatments.

Method History
Various methods for the determination of Triglycerides in human serum are currently in use in the routine clinical laboratory. With the advent of stable, more specific lipolitic enzymes, simpler and more efficient procedures have been described. (1,2) The Catachem Triglycerides method described below uses glycerophosphate oxidase coupled with a sensitive colorimetric endpoint reaction based on the work of Trinder. (3)

Method Principle
Serum Triglycerides are hydrolyzed by microbial lipase to glycerol and free fatty acids. The resultant glycerol, in the presence of glycerol kinase (GK) ATP and Mg^{++} ions is phosphorylated to glycerol-1-phosphate. This latter metabolite is then oxidized by glycerophosphate oxidase (GPO) to produce hydrogen peroxide. The hydrogen peroxide thus produced is quantitatively determined by coupling 4-aminoantipyrine with 4-chlorophenol where a quinonemine dye with maximum absorption at 505 nm is produced. The following reaction scheme illustrates the reactions that occur in this method:

$$\text{Triglycerides} \xrightarrow{\text{Lipase}} \text{glycerol + free fatty acids}$$

$$\text{Glycerol + ATP} \xrightarrow[Mg^{++}]{\text{GK}} \text{1-glycero-phosphate + ADP}$$

$$\text{1-glycerophosphate + } 0_2 \xrightarrow{\text{GPO}} H_20_2 + \text{dihydroxy acetone phosphate}$$

$$H_20_2\text{+4-chlorophenol +4AA} \xrightarrow{\text{peroxidase}} \text{quinoneimine dye + } 2H_20$$

Reagent Content
The concentration of the active ingredients in the reagent will be approximately as follows:

Triglycerides GPO-Trinder Reagent
One liter **R1** contains:

Buffer	
ATP	0.30 mM
4-aminoantipyrine	0.15 mM
4-chlorophenol	4.00 mM
Lipase	2000 U
Glycerol Kinase	1000 U
Glycerophosphate oxidase	500 U
Peroxidase	2000 U

Precautions
Avoid contact of the reagent with skin and eyes. Should contact occur, wash affected area with plenty of cold water. **DO NOT PIPETTE REAGENTS BY MOUTH**

Preparation Of Working Reagent
Catachem Triglycerides Reagent is packaged as a single liquid No preparation is required.

Reagent Storage And Stability
Store the reagents at 2-8°C. When stored as directed, these reagents are stable until the expiration date stated on the label. Upon opening and if stored capped when not in use, each TG Reagent component is stable for 60 days at 2-8°C.

Specimen Collection And Preparation
The use of clear, unhemolyzed serum is recommended. A fasting specimen is necessary for an accurate triglycerides determination.

Interfering Substances
A number of substances have been reported to affect the accuracy of triglycerides methods using the oxidase-peroxidase procedures (3,2). A summary of the influence of drugs on clinical laboratory procedures may be found by consulting D.S. Young, et al (5).

Expected Values
The "risk values" for Triglycerides in a human population as defined by the National Heart and Lung Institute are listed below. Levels in animals differ species to species.

AGE (Years)	**TRIGLYCERIDES (mg/dL)**
20-29	**140**
30-49	**160**
50-59	**190**

It is recommended that each laboratory establish the normal ranges for the species being tested and the species location.

Directions For Use
The Catachem Triglycerides Method requires one single reagent. Upon opening, the Working Reagent is stable for a minimum 60 days when stored capped at 2-8°C.

Procedure
Important: Read the entire instructions procedure before proceeding with the assay.

Materials Required But Not Provided
Spectrophotometer
Cuvettes 1 cm light path
Timer to time incubation time
Pipette 1.0 ml for reagent
Pipette 0.010 ml for sample

Materials Provided
Catachem Triglycerides Reagent

Analytical Parameters

Wavelength	505 nm
Temperature	37˚C
Pathlength	1 cm
Reaction Mode	Endpoint
Reaction Time	5 min
Reagent Volume R1	0.9 ml
Reagent Volume R2	0.1 ml
Sample Volume	0.01 ml (10µL)
Total Volume	1.01 ml
Sample-to-Reagent Ratio	1:100

Assay Procedure
1. Pipette 1 ml of Catachem Triglycerides Reagent into 3 separate cuvettes labeled “Calibrator”, "Sample”, and “Blank”

2. Pipette 0.01 ml (10 uL) of Calibrator or Sample into their respective cuvettes. Mix all cuvettes well.
3. Incubate all cuvettes for 5 minutes at 37˚C.
4. Set spectrophotometer wavelength at 505 nm and zero the instrument with the cuvette marked “Blank”.
5. Read the “Calibrator” and “Sample” absorbencies.
6. Calculate the Triglycerides concentration (mg/dL) in the Sample(s), as shown in “results and calculations” below.

Results And Calculations

$$\text{Triglycerides (mg/dL)} = \frac{\text{Sample Abs}}{\text{Calibrator Abs}} \times \text{Calibrator (mg/dL)}$$

Example:
Sample absorbance = 0.300
Calibrator absorbance = 0.250
Calibrator (mg/dL) = 200

$$\text{Triglycerides (mg/dL)} = \frac{0.300}{0.250} \times 200 = 240 \text{ mg/dL}$$

Quality Control
To monitor the performance of the Working Reagent and the procedure used, we recommend the regular use of a normal and abnormal control serum.

Method Performance Characteristics
Sensitivity: 0.0009-0013 absorbance units per mg/dL.
Linear Range: 0-1000 mg/dL.
Precision: Within-run and day-to-day precision is summarized below:

Triglycerides Precision Study

TGS	Within-Run		Total Precision	
Mean	SD	CV	SD	CV
mg/dL	mg/dL	%	mg/dL	%
65	1.0	1.5	1.6	2.2
103	0.5	0.5	1.7	1.6
390	1.2	0.3	3.0	0.8
722	3.8	0.6	7.9	1.1

Correlation
A comparison of the method using an automated analyzer and a reference method resulted in the following regression statistics:

Range	=	30-722 mg/dL
N	=	118
Y	=	1.03-5.6
r	=	0.997
Sy.x	=	7.0

References

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2. Fossati P, Prencipe L. Clin Chem 28, 10 (1980).
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5 .Trinder P, Ann Clin Biochem 6, 24 (1969).
4. Katsumi Tamaoku, Keiuy Ueno, Kayoko Akiura and Yosuke Ohkura. Chem PharmBull 30 (7) 2492-2497 (1982).
5. Young DS, Pestaner LD, Gibberman V. Clin Chem 21, 5 (1975).

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