



TRIGLYCERIDES

(LIQUID)

4 x 60 ml

RE – ORDER TRG1400

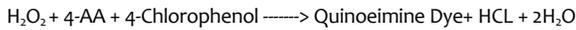
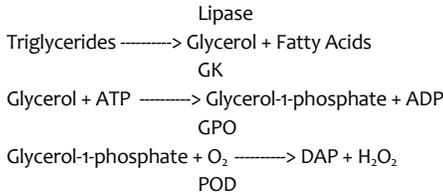
INTENDED USE

For the quantitative determination of triglycerides in serum or plasma.

METHOD AND HISTORY

The triglycerides (GPO) method is based on the enzymatic determination of glycerol using the enzyme glycerol phosphate oxidase (GPO) after hydrolysis by lipoprotein lipase. The principle of this method was described by Fossati who coupled the reaction with the classical Trinder reaction sequence. This single reagent procedure quantitates the total glycerides in serum including the mono and diglycerides, and the free glycerol fractions.

TEST 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 converted to glycerol-1-phosphate. The glycerol-1-phosphate is then oxidized by glycerol phosphate oxidase (GPO) to yield hydrogen peroxide. The condensation of hydrogen peroxide with 4-chlorophenol and 4-aminophenazone (4-AA) in the presence of peroxidase (POD) produces a red colored quinoneimine dye which absorbs at, or near 500nm. The intensity of the colored complex formed is directly proportional to the triglycerides concentration of the sample.

CLINICAL SIGNIFICANCE

Triglyceride determinations are of interest in the diagnosis and treatment of atherosclerosis, poorly controlled diabetes mellitus, nephrosis, liver disease, or other diseases involving lipid metabolism.

PATIENT PREPARATION

The patient should be fasting 12 hours prior to specimen collection.

SPECIMEN COLLECTION

Fresh clear, unhemolyzed serum is the preferred specimen. The specimen should be collected following the guidelines of NCCLS document H4-A3. The serum should be collected following a 12-hour fast and separated from the clot as soon as possible. Collection must be glycerol free. Glycerol can lead to falsely elevated results. Avoid blood collection tubes, which use glycerol to lubricate rubber stoppers. Avoid anticoagulants containing fluoride and oxalate. Detergents can interfere with action of lipase. Care should be taken to avoid contamination of laboratory equipment with detergents. Use a standard venipuncture tube to draw patient sample. The amount of sample required will depend on the analyzer used. The amount of serum required is in the range of 5–25ul. Call King's technical service department at 1-800-262-8655 for the recommended sample volume for your analyzer. Record the patient's name, date and time of sample collection and preparation.

SPECIMEN STORAGE

Specimens may be stored at 2-8°C (refrigerated) for one week and at -20°C (frozen) for 3 months. Thawed samples should not be refrozen. It is recommended that testing be done as soon as possible after sample collection and preparation. If testing cannot occur immediately, store the sample properly using the guidelines above.

REAGENT

Buffer (pH 7.4)		100 mM
4-Chlorophenol	3.5 mM	
ATP		0.6 mM
Magnesium acetate	12 mM	
4-Aminophenazone	0.3 mM	
Glycerol Kinase (microbial)	>250 U/L	
Glycerol Phosphate Oxidase (microbial)	>4500 U/L	
Peroxidase (horseradish)		>2000 U/L

Lipase (microbial) >200,000 U/L
Sodium azide 0.1%

Surfactants, Stabilizers, preservatives

WARNINGS AND PRECAUTIONS

This reagent set is for in vitro diagnostic use only.

This reagent contains sodium azide (0.1%) as a preservative. Do not ingest avoid skin and eye contact. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large amounts of water. All specimens and controls should be handled as potentially infectious.

REAGENT PREPARATION

The reagent is ready to use.

REAGENT STORAGE AND STABILITY

Store the reagent at 2 - 8°C. Unopened vials are stable till the expiration date when stored at 2 - 8°C. The reagent should not be used if: The reagent is turbid or displays evidence of bacterial contamination. The reagent blank is 0.350 or greater when measured at 500nm against water in a cuvette with one centimeter path length.

ADDITIONAL MATERIALS REQUIRED

Spectrophotometer capable of reading at 500nm.

Accurate pipetting devices for delivering required sample and reagent volumes.

Test tubes and racks.

Timer.

Heating block or water bath. (37°C)

TEST PROCEDURE

The following is a general procedure that can be used on a manual analyzer.

Application procedures for use on an automated analyzers are available.

PROCEDURE CONDITIONS

Wavelength	500nm
Temperature	37°C
Assay Type	Endpoint
Sample/Reagent Ratio	1:100
Reaction Direction	Increasing
Incubation	300 seconds

INSTRUMENT

Any instrument capable of reading absorbance accurately with a sensitivity of 0.001 absorbance at 500nm may be used. The band width should be 10nm or less, stray light 0.5% or less, and the wavelength accuracy within 2nm.

CALIBRATION

The triglyceride assay is calibrated by referencing the absorbance of the unknown sample to the absorbance of the calibrator.

PROCEDURE

Label test tubes: "Reagent Blank", "Standard", "Controls", "Unknowns", etc. Pipette 1.0 ml of reagent into all tubes. Pre-warm all tubes at 37°C. Add 0.01ml (10µl) of sample to respective tubes and swirl gently.

Incubate tubes at 37°C for five (5) minutes. After incubation, zero the spectrophotometer with the reagent blank at 500nm (500 – 520nm is acceptable) and read/record the absorbances of all tubes. The final color is stable for at least 60 minutes.

CALCULATION AND RESULTS

$$\begin{aligned} & A \\ \text{Triglyceride (mg/dl)} & = \frac{A}{A_s} \times \text{concentration of calibrator (mg/dl)} \end{aligned}$$

A = absorbance of sample, A_s = absorbance of calibrator

Example:

$$\begin{aligned} & .160 \\ \text{Triglyceride (mg/dl)} & = \frac{.160}{.265} \times 200 \text{ mg/dl} = 121 \text{ mg/dl} \end{aligned}$$

with A = .160 and A_s = .265, concentration of calibrator = 200 mg/dl

To obtain results in SI units (mmol), multiply your results in mg/dl by 0.0113.

EXPECTED VALUES

44-148 mg/dl (0.50-1.67 mmol/L)

Due to a wide range of conditions (dietary, geographical, age, etc.) believed to affect normal ranges, it is recommended that each laboratory establish its own reference range.

MEDICAL ALERT VALUES

Each laboratory should establish low and high values beyond which the patient would require immediate attention by a physician. If a "medical alert value" is reached, always repeat the test to confirm the result and notify a physician if the result is confirmed.

LIMITATIONS OF PROCEDURE

The procedure is linear to 1000 mg/dl. Specimens above 1000 mg/dl should be diluted 1:1 with saline and re-assayed. Multiply the result by 2 to compensate for the dilution. A number of drugs and substances affect the determination of triglycerides. (10.6,10.7) Young et al (10.8) have published a comprehensive list of substances. The method is not influenced by hemoglobin values up to 100mg/dl or by bilirubin levels up to 12mg/dl (<5%) Detergents can interfere with the action of lipase. Care should be taken to avoid contamination of laboratory equipment with detergents.

QUALITY CONTROL

Standard practice for Quality Control should be applied to this procedure. Commercially available controls (2 levels) should be used to monitor the daily acceptable variations. Controls should be assayed at the beginning of each shift, whenever a new lot number of reagent is used, or following any instrument maintenance. A satisfactory level of performance is achieved when the analyte values obtained are within the acceptable range established by the laboratory.

CALIBRATION PROCEDURES

Use King's serum based calibrator kit (catalog number 80194.)

The Triglyceride assay is calibrated by referencing the absorbance of the unknown sample to the absorbance of the calibrator. Refer to your instrument manual for more details. Calibration is required with the use of a new lot of reagent, any system maintenance or whenever indicated by quality control data.

PRECISION

Precision studies were performed following a modification of the guidelines which are contained in NCCLS document EP5-T2 (10.10).

Within-Run

Mean (U/L)	SD (U/L)	CV (%)
62.6	1.14	1.82
162.4	1.73	1.07
301.9	3.24	1.07

Run to Run

Mean (U/L)	SD (U/L)	CV (%)
59.1	1.12	1.90
158.1	2.63	1.66
299.3	3.65	1.22

CORRELATION

A correlation was made between this method and a similar GPO method using 167 samples ranging from 41 mg/dl to 1026 mg/dl. The correlation coefficient was 0.999 and the linear regression equation was $y = .97x - 4.5$. ($S_y - x = 5.84$)

LINEARITY

This procedure is linear to 1000 mg/dl beyond which the specimen should be diluted 1 to 1 with de-ionized water. Re-assay the specimen and multiply the result by 2.

REFERENCES

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