TRIGLYCERIDES

OSR60118	**4 x 20 mL**	**R1**
	4 x 5 mL	**R2**
OSR61118	**4 x 50 mL**	**R1**
	4 x 12.5 mL	**R2**
***OSR66118**	**4 x 167 mL**	**R1**
	4 x 43 mL	**R2**

Intended Use
System reagent for the quantitative determination of Triglyceride concentrations in human serum and plasma on Beckman Coulter AU analyzers.
*Triglycerides reagent OSR66118 for use on the AU2700/5400 system only.

Summary
Triglycerides are the major form of fat found in nature and their primary function is to provide energy for the cell.[1] Measurements of triglyceride are used in the diagnosis and treatment of patients with diabetes mellitus, nephrosis, liver obstruction, other diseases involving lipid metabolism, or various endocrine disorders.[2]

Clinically, triglyceride assays are used to help classify the various genetic and metabolic lipoprotein disorders and in the assessment of risk factors for atherosclerosis and coronary artery disease.[3,4]

Methodology
This Triglyceride procedure is based on a series of coupled enzymatic reactions.[5,6] The triglycerides in the sample are hydrolyzed by a combination of microbial lipases to give glycerol and fatty acids. The glycerol is phosphorylated by adenosine triphosphate (ATP) in the presence of glycerol kinase (GK) to produce glycerol-3-phosphate. The glycerol-3-phosphate is oxidized by molecular oxygen in the presence of GPO (glycerol phosphate oxidase) to produce hydrogen peroxide (H_2O_2) and dihydroxyacetone phosphate. The formed H_2O_2 reacts with 4-aminophenazone and N,N-bis(4-sulfobutyl)-3,5-dimethylaniline, disodium salt (MADB) in the presence of peroxidase (POD) to produce a chromophore, which is read at 660/800nm. The increase in absorbance at 660/800 nm is proportional to the triglyceride content of the sample.

$$\text{Triglycerides} + 3\,H_2O \xrightarrow{\text{Lipase}} \text{Glycerol} + 3\,\text{Fatty Acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK, } Mg^{2+}} \text{Glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{GPO}} H_2O_2 + \text{Dihydroxyacetone phosphate}$$

$$2\,H_2O_2 + \text{MADB} + \text{4AAP} \xrightarrow{\text{Peroxidase}} \text{Blue Dye} + OH^- + H_2O$$

System Information
For AU400/400^e/480, AU600/640/640^e/680 and AU2700/5400 Beckman Coulter Analyzers.

Reagents
Final concentration of reactive ingredients:

PIPES buffer (pH 7.5)	50 mmol/L
Lipase (Pseudomonas)	≥ 1.5 kU/L (25 µkat/L)
Glycerol kinase (Bacillus stearothermophilus)	≥ 0.5 kU/L (8.3 µkat/L)
Glycerol phosphate oxidase (Pseudomonas)	≥ 1.5 kU/L (25 µkat/L)
Ascorbate oxidase (Curcubita species)	≥ 1.5 kU/L (25 µkat/L)
Peroxidase (Horseradish)	≥ 0.98 kU/L (16.3 µkat/L)
ATP	1.4 mmol/L
4-Aminoantipyrine	0.50 mmol/L
Magnesium acetate	4.6 mmol/L
MADB	0.25 mmol/L

Also contains preservatives.

Precautions
1. For *in vitro* diagnostic use.
2. WARNING! POISON! Contains phenol which may be harmful if swallowed. Avoid contact with eyes, skin and mucous membranes. In case of contact, immediately rinse affected area with large amounts of water.
3. Contains sodium azide as a preservative which may react with lead joints in copper plumbing to form explosive compounds. Even though the reagent contains minute quantities of sodium azide, drains should be well flushed with water when discarding the reagent.
4. Dispose of all waste material in accordance with local guidelines.

Triglycerides

Preparation of Reagents
For OSR60118 and OSR61118, the Triglyceride Reagents are ready for use. No preparation is required. For OSR66118, insert the pipe supplied into the 180 mL reagent vial before use on the analyzer. Care must be taken when handling the pipe to avoid contamination. The pipe is for single use only.

Storage and Stability
1. The unopened reagents are stable until the expiration date printed on the label when stored at 2 – 8°C.
2. Opened reagents are stable for 30 days when stored in the refrigerated compartment of the analyzer.

Indications of Deterioration
Visible signs of microbial growth, gross turbidity, precipitate or change in color in the Triglyceride reagent may indicate degradation and warrant discontinuance of use.

Specimen Collection and Preparation

Fasting (≥ 12 hours) serum samples,[7] free from hemolysis and removed from the clot are the recommended specimens. EDTA and heparin are the suggested anticoagulants if plasma must be used.
Ensure that all equipment used in the collection and storage of samples is free from glycerol contamination.

Sample Storage and Stability
Serum triglyceride is stable for seven days when stored at 2 - 8°C and 3 months when stored frozen at ≤ -20°C.[8]

Interfering Substances
Results of studies[9] show that the following substances interfere with this triglyceride procedure.

The criteria for no significant interference is recovery within 10% of the initial value.
Ascorbate: No significant interference up to 20 mg/dL Ascorbate
Bilirubin: No significant interference up to 40 mg/dL Bilirubin
Hemolysis: No significant interference up to 500 mg/dL Hemolysate

The information presented is based on results from Beckman Coulter studies and is current at the date of publication. Beckman Coulter Inc. makes no representation about the completeness or accuracy of results generated by future studies. For further information on interfering substances, refer to Young[10] for a compilation of reported interferences with this test.

Procedure

A complete list of test parameters and operational procedure can be found in the User's Guide appropriate to the analyzer.

Materials Provided
Triglyceride Reagent
Pipe (one per each 180 mL vial)

Materials Required But Not Provided
Chemistry Calibrator (Cat # DR0070)

Stability of the Final Reaction Mixture
The Beckman Coulter AU analyzer automatically computes every determination at the same time interval.

Calibration
The frequency of calibration is every 30 days. Calibration of the Triglyceride procedure is accomplished by use of Chemistry Calibrator (Cat # DR0070), which is traceable to the College of American Pathology (CAP) Serum Lipid (RM016) #2.
Recalibration of this test is required when any of these conditions exist:
1. A reagent lot number has changed or there is an observed shift in control values.
2. Major preventative maintenance was performed on the analyzer.
3. A critical part was replaced.

Quality Control
During operation of the Beckman Coulter AU analyzer, at least two levels of an appropriate quality control material should be tested a minimum of once a day. In addition, controls should be performed after calibration with each new lot of reagent, and after specific maintenance or troubleshooting steps described in the appropriate User's Guide. Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure.

Results
Results are automatically printed out for each sample in mg/dL at 37°C. For SI Units (mmol/L) the results must be multiplied by 0.0113.

Dynamic Range

This Triglyceride procedure is linear from 10 to 1000 mg/dL. Samples exceeding the upper limit of linearity should be diluted and repeated. The sample may be diluted, repeated and multiplied by the dilution factor automatically utilizing the AUTO REPEAT RUN.

Note: Triglycerides GPO enzymatic methodologies are subject to a strong negative interference from patient samples with extremely elevated triglyceride levels.[11] While these samples are extremely lipemic in appearance and typically have triglyceride levels exceeding 1700 mg/dL, results can be erroneously reported as being within the linear range of the assay. In order to identify grossly lipemic samples exhibiting this phenomenon, Data Check Parameters are provided. If the reaction kinetics of a test exhibits the characteristics of one of these elevated triglyceride samples, the analysis result will be flagged (F, Z, @ or &). Grossly lipemic samples under rare circumstances may evade the Data Check Parameters and should routinely be diluted 1 part sample to 4 parts saline prior to analysis and the results multiplied by 5.

Expected Values

Triglyceride	Risk Classification[12]
<150 mg/dL	Normal
150-199 mg/dL	Borderline High
200-499 mg/dL	High
≥500 mg/dL	Very High
Adults[13]	48 - 352 mg/dL

Expected values may vary with age, sex, diet and geographical location. Each laboratory should determine its own expected values as dictated by good laboratory practice.

Specific Performance Characteristics

The following data was obtained using the Triglyceride Reagent on Beckman Coulter AU analyzers according to established procedures. Results obtained in individual laboratories may differ.

Precision[15]

Estimates of precision, based on CLSI recommendations,[14] are consistent with typical performance. The within run precision is less than 3% CV and total precision is less than 5% CV. Assays of serum pools were performed and the data reduced following CLSI guidelines above.

N = 80	Within run		Total	
Mean, mg/dL	SD	CV%	SD	CV%
89.4	0.57	0.64	1.48	1.65
191	0.95	0.49	2.69	1.41
442	2.28	0.51	6.45	1.46

Method Comparison[15]

Patient samples were used to compare this Triglyceride Reagent. The table below demonstrates representative performance on AU analyzers.

Y Method	AU640
X Method	Method 2
Slope	1.011
Intercept	-0.871
Correlation Coeff. (r)	1.000
No. of Samples (n)	148
Range (mg/dL)	14 - 939

Analytical Sensitivity (Lower Detection Limit)

The lowest detectable level using serum settings on an AU analyzer was calculated as 0.31 mg/dL.

The lowest detectable level represents the lowest measurable level of triglyceride that can be distinguished from zero. It is calculated as the absolute mean plus three standard deviations of 20 replicates of an analyte free sample.

Limit of Quantitation

The Limit of Quantitation (LOQ) using serum settings for the Triglyceride reagent was determined to be 5 mg/dL. This was determined according to CLSI protocol EP17-A[16] and represents the lowest concentration of triglyceride that can be measured with a total imprecision of 20%.

References

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