BECKMAN COULTER CE

AU

Instructions For Use

TRIG
TRIGLYCERIDE

REF 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 173 mL R1, 4 x 48 mL R2

For *in vitro* diagnostic use only.

PRINCIPLE

INTENDED USE

Enzymatic colour test for the quantitative determination of triglyceride in human serum and plasma on Beckman Coulter AU analysers.

OSR66118 for use on the AU5800, AU2700 and AU5400 systems only.

SUMMARY AND EXPLANATION

Reference[1,2]

Measurements of triglyceride are used in the diagnosis and treatment of patients with acute and chronic pancreatitis, diabetes mellitus, nephrosis, extrahepatic biliary obstruction, and other diseases involving lipid metabolism, or various endocrine disorders.

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

METHODOLOGY

Reference[3,4,5]

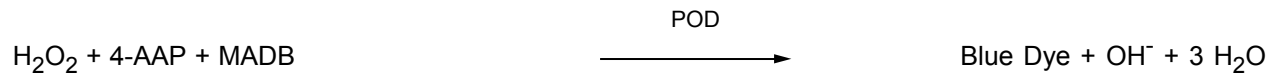
This Triglyceride procedure is based on a series of coupled enzymatic reactions. The triglycerides in the sample are hydrolysed by a combination of microbial lipases to give glycerol and fatty acids. The glycerol is phosphorylated by adenosine triphosphate (ATP) in the presence of glycerol kinase (GK) to produce glycerol-3-phosphate. The glycerol-3-phosphate is oxidised 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 660/800nm is proportional to the triglyceride content of the sample.

CHEMICAL REACTION SCHEME

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

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

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



SPECIMEN

TYPE OF SPECIMEN

Reference[1]

Serum and EDTA or heparinised plasma.

Plasma using anticoagulants such as fluoride, citrate and oxalate should be avoided.

Strongly icteric samples should be avoided.

Avoid using vacuum tubes with glycerol-coated stoppers.

Stable in serum and plasma for 7 days when stored at 2…8°C and 2 days when stored at 15…25°C.[6]

REAGENTS

WARNING AND PRECAUTIONS

Exercise the normal precautions required for handling all laboratory reagents.

Dispose of all waste material in accordance with local guidelines.

This product contains material of animal origin. The product should be considered as potentially capable of transmitting infectious diseases.

REACTIVE INGREDIENTS

Final concentration of reactive ingredients:

PIPES buffer (pH 7.5)	50 mmol/L	Lipases	1.5 kU/L (25 µkat/L)
Mg^{2+}	4.6 mmol/L	Glycerol kinase	0.5 kU/L (8.3 µkat/L)
MADB	0.25 mmol/L	Peroxidase	0.98 kU/L (16.3 µkat/L)
4-Aminoantipyrine	0.5 mmol/L	Ascorbate Oxidase	1.48 kU/L (24.6 µkat/L)
ATP	1.4 mmol/L	Glycerol-3-phosphate oxidase	1.48 kU/L (24.6 µkat/L)

Preservative

The concentrations of the reactive components of the reagents shown on the kit label are the actual concentrations in the individual R1/R2 vials. The reagent composition which is shown in the Instructions For Use is the final concentration of these components in the reaction cuvette after addition of R1, Sample, and R2.

⚠ CAUTION

Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76).
To avoid the possible build-up of azide compounds, flush wastepipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations.

GHS HAZARD CLASSIFICATION

Not classified as hazardous

SDS	Safety Data Sheet is available at beckmancoulter.com/techdocs

REAGENT PREPARATION

The reagents are ready for use and can be placed directly on board the instrument.

STORAGE AND STABILITY

The reagents are stable, unopened, up to the stated expiry date when stored at 2…8°C. Once open, reagents stored on board the instrument are stable for 30 days.

A very fine suspension of particles which may settle out on storage may be evident in the R1 component of this reagent. The reagent can be used without effect to results.

CALIBRATION

CALIBRATOR REQUIRED

System Calibrator Cat. No. 66300.

The calibrator triglyceride value provided in the calibrator package insert is traceable to the Isotope Dilution Mass Spectrometry Reference Method.

Recalibrate the assay every 30 days, or when the following occur:

Change in reagent lot number or significant shift in control values;

Major preventative maintenance was performed on the analyser or a critical part was replaced.

QUALITY CONTROL

Controls Cat. No. ODC0003 and ODC0004 or other control materials with values determined by this Beckman Coulter system may be used.

Each laboratory should establish its own control frequency however good laboratory practice suggests that controls be tested each day patient samples are tested and each time calibration is performed.

The results obtained by any individual laboratory may vary from the given mean value. It is therefore recommended that each laboratory generates analyte specific control target values and intervals based on multiple runs according to their requirements. These target values should fall within the corresponding acceptable ranges given in the relevant product literature.

If any trends or sudden shifts in values are detected, review all operating parameters.

Each laboratory should establish guidelines for corrective action to be taken if controls do not recover within the specified limits.

TESTING PROCEDURE(S)

Refer to the appropriate Beckman Coulter AU analyser User Guide/Instructions For Use (IFU) for analyser-specific assay instructions for the sample type as listed in the Intended Use statement. The paediatric application is suitable for use with small volume serum/plasma samples.

CALCULATIONS

The Beckman Coulter analysers automatically compute the triglyceride concentration of each sample.

REPORTING RESULTS

REFERENCE INTERVALS

Reference[7]	
Normal	< 1.70 mmol/L (150 mg/dL)
Borderline high	1.70 – 2.25 mmol/L (150 – 199 mg/dL)
High	2.26 – 5.64 mmol/L (200 – 499 mg/dL)
Very high	≥ 5.65 mmol/L (500 mg/dL)

Expected values may vary with age, sex, sample type, diet and geographical location. Each laboratory should verify the transferability of the expected values to its own population, and if necessary determine its own reference interval according to good laboratory practice. For diagnostic purposes, results should always be assessed in conjunction with the patient's medical history, clinical examinations and other findings.

Data contained within this section is representative of performance on Beckman Coulter systems. Data obtained in your laboratory may differ from these values.

PROCEDURAL NOTES

LIMITATIONS

Note: Triglycerides GPO enzymatic methodologies are subject to a strong negative interference from patient samples with extremely elevated triglyceride levels.[8] While these samples are extremely lipemic in appearance and typically have triglyceride levels exceeding 20 mmol/L, 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 exhibit 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.

If compensating for free glycerol, subtract 0.11 mmol/L (10 mg/dL) from the triglyceride value obtained.[1] This correction is mainly applicable to healthy individuals, but for some diseases, for example diabetes or liver disorders, a higher concentration of free glycerol may occur.

INTERFERENCES

Results of studies conducted to evaluate the susceptibility of the method to interference were as follows:

Ascorbate :	Interference less than 5% up to 20 mg/dL ascorbate
Icterus:	Interference less than 3% up to 40 mg/dL or 684 µmol/L bilirubin
Haemolysis:	Interference less than 3% up to 5 g/L haemoglobin

Venipuncture immediately after or during the administration of Metamizole (Dipyrone) may lead to falsely low results for Triglyceride. Venipuncture should be performed prior to the administration of Metamizole.

Refer to Young[9] for further information on interfering substances.

PERFORMANCE CHARACTERISTICS

LINEARITY

The test is linear within a concentration range of 0.1 – 11.3 mmol/L (10 – 1,000 mg/dL).

Prozone settings must be applied when using the Triglyceride reagent, refer to Setting Sheet for specific instrument details.

SENSITIVITY

The lowest detectable level in serum on an AU640 analyser was estimated at 0.01 mmol/L.

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.

METHODS COMPARISON

Patient serum samples were used to compare this Triglyceride OSR61118 assay on the AU640 against another commercially available triglyceride assay. Results of linear regression analysis were as follows:

y = 0.966x – 0.005	r = 1.000	n = 101	Sample range = 0.60 – 10.85 mmol/L

PRECISION

The following data was obtained on an AU640 using 3 serum pools analysed over 20 days.

n = 80	**Within-run**		**Total**	
Mean mmol/L	**SD**	**CV%**	**SD**	**CV%**
0.47	0.01	1.06	0.01	1.76
4.28	0.03	0.72	0.04	1.03
10.20	0.08	0.79	0.15	1.46

ADDITIONAL INFORMATION

DxC 700 AU requires that each reagent application has a standard format of abbreviated Closed Test Name. This Closed Test Name is required to allow automated loading of the calibrator information for each application as part of the DxC 700 AU Closed System. Refer to the table below for the Closed Test Name assigned to each application for this assay.

Test Name	**Description**
TRG1N	Triglyceride (Serum)
TRG1NP	Triglyceride (Serum Paediatric)

Setting Sheet Footnotes
User defined
† System Calibrator Cat. No.: 66300
* Values set for working in SI units (mmol/L). To work in mg/dL multiply by 88.5

REVISION HISTORY

Added new languages

Preceding version revision history

IFU updated to add Vietnamese language.

Updated Additional Information section

REFERENCES

1. Tietz NW, ed. Clinical guide to laboratory tests, 3rd ed. Philadelphia: WB Saunders Company, 1995: 610-611.
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5. Trinder P. Ann Clin Biochem, 1969; 6:24-27.
6. Guder, WG, Ehret W, Heil W, Schmitt Y, Töpfer G, Wisser H, Zawta B, et al. Use of anticoagulants in diagnostic laboratory investigations and stability of blood, plasma and serum samples. WHO/DIL/LAB/99.1 Rev.2: 44pp.
7. National Cholesterol Education Program Expert Panel. Executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001;285: 2486-2497.
8. Shephard MD, Whiting MJ. Falsely low estimation of triglycerides in lipemic plasma by the enzymatic triglyceride method with modified Trinder's chromogen. Clin Chem 1990; 36:325-329.
9. Young DS. Effects of drugs on clinical laboratory tests, 5thed. AACC Press, 2000.

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