TRIGLYCERIDES

Diagnostic reagent for determination of Triglycerides concentration.
Liquid. Monoreagent. Store at +2/+8°C. For in Vitro Diagnostic Use (IVD). Do not freeze.

Ref No	Pack
MH-272	200 mL
MH-273	120 mL

Changes made in the instructions for use are marked as grey.

INTENDED USE

The Archem triglyceride test is used for the quantitative in vitro determination of triglyceride concentration in human serum or plasma by autoanalyzers in a clinical laboratory setting.

GENERAL INFORMATION

The simplest lipids composed of fatty acids are triacylglycerols (TAGs), also known as triglycerides (TG), fats or neutral fats. TG consists of three fatty acids, each linked to a single glycerol by an ester bond.[1] More than 90% of the daily lipid intake by adults is TG. The rest of the dietary lipids consist mainly of cholesterol, cholesteryl esters, phospholipids and non-esterified ("free") fatty acids. Digestion of lipids begins in the stomach, catalyzed by a lipase (lingual lipase) that originates from glands on the back of the tongue. TG molecules, especially those containing fatty acids of short or medium chain length (less than 12 carbons, as found in milk fat) are the primary target of this enzyme. Similar TGs are also broken down by a separate gastric lipase secreted by the gastric mucosa. The major absorption of TG molecules occurs by the mucosal cells of the intestinal villi and prior to the absorption, it is hydrolyzed by an esterase enzyme called pancreatic lipase to remove fatty acids, preferably at carbons 1 and 3. As a result, the main products of hydrolysis are 2-monoacylglycerol and free fatty acids.[2] As a result, dietary TGs are absorbed from the small intestine and secreted into the lymphatic system. They then pass into the systemic circulation as chylomicrons (CMs) via the ductus thoracicus. Muscle and adipose tissue separate some of the TGs from the chylomicrons and then, the liver removes the chylomicron residues and converts them into cholesterol-rich lipoproteins. Although most of the TGs in the blood are absorbed from the small intestine, the liver also produces and releases some TGs into the blood. Apolipoproteins are proteins that help in the binding, transport and metabolism of lipids and also interact with TGs.

Damage to the structure of these proteins or related enzymes may lead to clinical dyslipidemia.[3]

Specialized cells called adipocytes or fat cells store large amounts of TGs in the form of fat droplets that almost fill the cell. When energy is needed, the TGs stored in adipocytes are hydrolyzed by lipase enzymes and transported as free fatty acids to sites in the body where they are needed as fuel. An advantage of using TGs instead of polysaccharides such as glycogen and starch as stored fuel is that the oxidation of TGs yields twice as much energy as the oxidation of carbohydrates, since the carbon atoms of fatty acids are more reduced than those of sugars. Humans have adipose tissue under the skin, in the abdominal cavity and in the mammary glands. With 15 to 20 kg of TGs accumulated in their adipocytes, moderately obese people can meet their energy needs for months by using their fat stores. Most natural fats, such as vegetable oils, dairy products and animal fats, contain complex mixtures of simple and mixed TGs. These foods contain a variety of fatty acids that differ in chain length and degree of saturation. For example, vegetable oils, such as corn and olive oil, are composed of TGs that contain largely unsaturated fatty acids and are therefore liquid at room temperature.[1] Hypertriglyceridemia is a disorder of lipid metabolism resulting from excessive synthesis, defective processing and clearance, or both. The fact that hypertriglyceridemia has recently been identified as a risk factor for the development of cardiovascular disease (CVD) and has been known to play an important role in the etiopathogenesis of acute non-biliary pancreatitis and non-alcoholic steatohepatitis for years, reveals the necessity of its treatment. In recent prospective studies, the link between hypertriglyceridemia and CVD has been reported especially in Type 2 diabetics with low HDL-C and high LDL-C levels. In the presence of severe hypertriglyceridemia, eruptive xanthomas and lipemia retinalis may be seen in addition to acute pancreatitis.

In some cases, very high levels of CM can cause chylomicronemia syndrome, characterized by recurrent abdominal pain, nausea, vomiting and pancreatitis.

If a secondary cause cannot be identified, the disorder is classified as primary hypertriglyceridemia. Many genetic defects in TG metabolism may be responsible for primary hypertriglyceridemia. Ethnic factors may also play an important role in the development of dyslipidemia. Although primary causes account for a very small proportion of hypertriglyceridemia, they should be considered in cases of severe hypertriglyceridemia and family history should be questioned. Although molecular studies are required for definitive diagnosis, molecular studies can be performed in 5% of all cases. The main diagnosis is based on clinical and family history. The main primary causes are mixed hypertriglyceridemia (Type V hyperlipoproteinemia), familial

hypertriglyceridemia (Type IV hyperlipoproteinemia), familial combined dyslipidemia and familial dysbetalipoproteinemia (Type III hyperlipoproteinemia). The main causes of secondary hypertriglyceridemia are obesity, diabetes mellitus, hypothyroidism, nephrotic syndrome, sedentary life, some drugs (beta-blockers, glucorticoids, estrogen, some immunosuppressive drugs, thiazide group diuretics, etc.) and some other diseases (Cushing's syndrome, rheumatoid arthritis, systemic lupus erythematosus, AIDS, Gaucher disease, Werner syndrome, sepsis, etc.).[3]

TEST PRINCIPLE

Colorimetric enzyme method

TGs are enzymatically hydrolyzed to fatty acids and glycerol by lipoprotein lipase. Glycerol is phosphorylated by adenosine triphosphate (ATP) to form glycerol-3-phosphate (G-3-P) and adenosine diphosphate (ADP) under the catalysis of the enzyme glycerol kinase (GK). G-3-P is oxidized to dihydroxyacetone phosphate (DAP) by glycerol phosphate oxidase (GPO), producing hydrogen peroxide (H_2O_2). H_2O_2 reacts with 4-chlorophenol (4-CP) and 4-aminoantipyrine (4-AAP) in the presence of peroxidase to form a red dye called quinoneimine. The color intensity is proportional to the concentration of TG and is measured photometrically.

REAGENT COMPONENTS

4-chlorophenol	: 2.7 mM
4-AAP	: 0.3 mM
ATP	: 2 mM
GK	: > 1000 U/L
POD	: > 1000 U/L
LPL	: > 2000 U/L
GPO	: > 5000 U/L
Good's buffer pH 7.20	: 50 mM
Surfactants	

REAGENT PREPARATION

Reagent is ready for use.

REAGENT STABILITY AND STORAGE

Reagents are stable at +2/+8°C till the expiration date stated on the label which is only for closed vials.

Once opened vials are stable for 60 days at +2/+8°C in optimum conditions. On board stability is strongly related to auto analyzers' cooling specification and carry-over values.

Reagent stability and storage have been verified by using Clinical and Laboratory Standards Institute (CLSI) EP25-A protocol.[4]

SAMPLE REQUIREMENTS

Serum and plasma can be used and are collected according to the standard procedures. Serum and plasma can be used and are collected according to the standard procedures. For plasma, sample collection tubes with Li heparin should be preferred. Sample collection tubes with EDTA must not be preferred for plasma. Hemolyzed samples must not be used.

Triglycerides activity stability in serum and plasma[16]
    2 days at +20/+25°C
    7 days at +2/+8°C
    1 year at -20°C

Unit Conversion:
mg/dL x 0.0113 = mmol/L

CALIBRATION AND QUALITY CONTROL

Calibration: The assay requires the use of an Arcal Auto Calibrator. We recommend:

Arcal Auto Calibrator
Ref.No: VT-003

Calibration stability depends on the application characteristics and cooling capacity of the autoanalyzer used. Calibration stability is 30 days.

Control: Commercially available control material with established values determined by this method can be used. We recommend:

Arcon N (Level I Control) Lyophilized
Ref.No: VT-001

Arcon P (Level II Control) Lyophilized
Ref.No: VT-002

Traceability is provided by ACS Grade material.

At least two level controls must be run once in every 24 hours. Each laboratory should determine its own quality control scheme and procedures. If quality control results are not within acceptable limits, calibration is required.

REFERENCE INTERVAL / MEDICAL DECISION LEVELS

Serum triglyceride levels are assessed according to the risk of CVD:

Serum/Plazma[17]:

		mg/dL	mmol/L
•	Normal	: <150 mg/dL	<1.70 mmol/L
•	Borderline High	: 150-199 mg/dL	1.70-2.25 mmol/L
•	High	: 200-499 mg/dL	2.26-5.64 mmol/L
•	Very High	: ≥ 500 mg/dL	≥ 5.65 mmol/L

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary, determine its own reference range.[5]

Note: Triglycerides should be measured after 12-16 hours of fasting. The NCEP-ATP has set the age of fasting lipid panel (LDL-C, HDL-C and TG) assessment age at 20 and recommends that it be repeated every 5 years. If there is a significant risk factor, this should be repeated every year. If the TG value is above 150 mg/dL, the test can be repeated after a fast. If available, apolipoprotein B and Lipoprotein (a) measurements can

be performed to determine the risk of atherosclerosis in the presence of hypertriglyceridemia.[3]

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary, determine its own reference range.

Reference interval has been verified by using Clinical and Laboratory Standards Institute (CLSI) EP28-A3c protocol.[6]

PERFORMANCE CHARACTERISTICS

Measuring Interval
According to CLSI EP34-ED1:2018, "Measuring Interval" refers to the interval where the analyte concentration is measured with intended accuracy in terms of medical and laboratory requirements without dilution, concentrating or any kind of pre-treatment that is between the analyte's lower limit of quantitation (LLoQ) and upper limit of quantitation (ULoQ).[7]

The determined analytic measuring interval for Triglycerides: 10-1000 mg/dL.

Detection Capability

Limit of Detection (LoD): 5 mg/dL

Limit of Quantitation (LoQ): 10 mg/dL

Note: LoQ values are based on Coefficient of Variation Percentage (CV) ≤ 20%.

LoD and LoQ values have been verified by using CLSI EP17-A2:2012 protocol.[8]

Linearity
This method shows measurement linearity in the activities up to 1000 mg/dL.
Autoanaylzer's auto-dilution system can be used if the concentrations have higher values. See device manual for further information.

For manual dilution procedure, dilute the sample 10-fold using 0.9% isotonic. After the dilution, multiply the result of rerun sample by the dilution factor. Do not report the sample result after dilution if it is marked as lower than the linear lower limit. Rerun with a suitable dilution.

Linearity studies data have been verified by using CLSI EP06-A:2003 protocol.[9]

Precision
Running system has been developed according to 20x2x2 "The Single Site" protocol. Repeatability and Within-Laboratory Precision / Within-Device values have been obtained according to the running results.

According to the protocol in use, 2 separate runs per day have been made for 20 days (no obligation for being consecutive days). This protocol has been applied to each low and high samples separately and 80 results have been obtained for each one. Statistically, the results have been obtained using 2-factor Nested-ANOVA model.[10]

Repeatibility (Within Run) and Repeatibility (Day to Day) SD (standard deviation) and CV% values of Triglycerides have been given in the table 1 and 2 respectively.

Table 1. Triglycerides Repeatability (Within Run) results obtained from samples in two different concentrations

Mean Concentration	SD*	%CV	n
97 mg/dL	1.37	1.41	80
185 mg/dL	3.12	1.68	80

Note: This working system has been named "Within-Run Precision" in the previous CLSI - EP05-A2 manual.[11]

Table 2. Triglycerides Repeatability (Day to Day) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
90 mg/dL	1.67	1.85	80
219 mg/dL	6.03	2.75	80

Note: This working system has been named "Total Precision" in the previous CLSI - EP05-A2 manual.[11]

Method Comparison
As a result of the statistical evaluation of the method comparison data:

Passing-Bablock equation:[12]
y= 1.01x+2.85 mg/dL
r=0.999

Interference
Endogenous interferant and analyte concentrations that have been used in the Triglycerides scanning tests has been determined according to "CLSI EP37-ED1:2018" and "CLSI EP07-ED3:2018" manuals.[13,14]
The total acceptable error rate, which is going to be used to detect whether the observed differential value obtained from Triglycerides interference scanning test is appropriate, is determined as ±25%.[15]

In Triglycerides test results, no significant interaction has been observed in the determined endogenous interferant and analyte concentrations or between interferants and analyte.

Interfering Substance and Concentration	Triglycerides Target (U/L)	N*	Observed Recovery %
Hemoglobin 180 mg/dL	137	3	107
Bilirubin 4,11 mg/dL	171	3	94

* Total acceptable error rate determined as interference limit and repeatability (within run) pre-detected for the related method were used for the calculations of how many times the control and test samples prepared as a serum pool are going to be run repetitively. In the calculations, the accepted error rate for type 1 (α error) was 5% and for type 2 (β error) was 10% (90% power). [14]

It should be noted that endogenous interferants, as well as various medicines and metabolites, anticoagulants (e.g. Heparin, EDTA, citrate, oxalate) and preservatives (e.g. sodium floride, iodoacetate, hydrochloride acide) such as additives, materials that may contact with samples during collection and processing (serum separator devices, sample collection containers and contents, catheters, catheter wash solutions, skin disinfectants, hand cleaners and lotions, glass washing detergents, powder gloves), dietary substances known to affect some specific tests (caffeine, beta-carotene, poppy seeds, etc.), or some substances present in a sample that cause foreign proteins (heterophilic antibodies, etc.), autoimmune response (autoantibodies, etc.), or due to malignancy (for example, interference by paraproteins with phosphate testing and indirect ion selective electrode methods) may show some negative effects that will cause various attempts and some misjudgements.[14]

These performance characteristics have been obtained using an autoanalyzer. Results may vary slightly when using different equipment or manual procedures.

WARNINGS AND PRECAUTIONS

IVD: For in Vitro Diagnostic use only.
Do not use expired reagents.
Reagents with two different lot numbers should not be interchanged.
For professional use.
Follow Good Laboratory Practice (GLP) guidelines.

CAUTION: Human source samples are processed with this product. All human source samples must be treated as potentially infectious materials and must be handled in accordance with OSHA standards.

Danger

EUH032	:Releases a very toxic gas if contacts with acid.
H317	:May cause allergic skin reaction.

Precaution

P280	:Use protective gloves / clothes / glasses / mask.
P264	:Wash your hands properly after using.
P272	:Contaminated work clothes should not be allowed to be used outside of the workplace.

Intervention

P302+P352	:Wash with plenty of water and soap if it contacts with skin.
P333+P313	:Seek medical help if it irritates your skin or develops rash.
P362+P364	:Remove contaminated clothes and wash properly before using.

Disposal

P501	:Dispose the vials and contents according to the local regulations.

REFERENCES

1. Nelson, D. R., & Cox, M. M., Lehninger-Principles of Biochemistry, Chapter 10: Lipids, p.343-68, Macmillan Learning.
2. Ferrier, D. R., (2014), Lippincott's Illustrated Reviews: Biochemistry (6th ed.), Chapter 14: Glycosaminoglycans, Proteoglycans, and Glycoproteins, p.157-72, Wolters Kluwer Health.
3. TEMD Obezite, Lipid Metabolizması ve Hipertansiyon Çalışma Grubu, (2015), Lipid Metabolizma Bozuklukları Tanı ve Tedavi Kılavuzu (1st ed.), Chapter 5: Trigliserid Yükekliğine Yaklaşım, p.25-28, Türkiye Endokrinoloji ve Metabolizma Derneği, 978-605-4011-23-0.
4. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline. CLSI Document EP25-A. Wayne, PA: CLSI; 2009.
5. Clinical and Laboratory Standards Institute (CLSI). Verification of Reference Intervals in the Medical Laboratory Implementation Guide – Third Edition. CLSI Document EP28 ED3IG. Wayne, PA: CLSI; 2022.
6. Clinical and Laboratory Standards Institute (CLSI). Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline – Third Edition. CLSI Document EP28-A3c. Wayne, PA: CLSI; 2010.
7. Clinical and Laboratory Standards Institute (CLSI). Establishing and Verifying an Extended Measuring Interval Through Specimen Dilution and Spiking – 1st Edition. CLSI Document EP34. Wayne, PA: CLSI; 2018
8. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition. CLSI Document EP17-A2. Wayne, PA: CLSI; 2012.
9. Clinical and Laboratory Standards Institute (CLSI). Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach - 1st Edition. CLSI Document EP06-A. Wayne, PA: CLSI; 2003.
10. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition. CLSI Document EP05-A3. Wayne, PA: CLSI; 2014.
11. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline - Second Edition. CLSI Document EP05-A2. Wayne, PA: CLSI; 2004.
12. Bablok W et al. A General Regression Procedure for Method Transformation. J Clin Chem Clin Biochem 1988;26:783-790.
13. Clinical and Laboratory Standards Institute (CLSI). Supplemental Tables for Interference Testing in Clinical Chemistry - First Edition. CLSI Document EP37. Wayne, PA: CLSI; 2018.

14. Clinical and Laboratory Standards Institute (CLSI). Interference Testing in Clinical Chemistry - Third Edition. CLSI Document EP07. Wayne, PA: CLSI; 2018.

15. CLIA proficiency testing criteria for acceptable analytical performance, as printed in the Federal Register July 11, 2022;87(131:41194-242.

16. Guder WG, Narayanan S, Wisser H, et al. List of analytes—preanalytical variables. Annex In: Samples: From the Patient to the Laboratory. Darmstadt, Germany: GIT Verlag; 1996:Annex 22–3

17. 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;285:2486–97

Archem Sağlık Sanayi ve Tic. A.Ş. (With official contract based manufacturing agreement with Validity Sağlık Hiz. Sanayi A.Ş. Company)
Mahmutbey Mah. Halkalı Cad. No:124 Kat:4 Bağcılar/İstanbul/Türkiye
Tel: + 90 212 444 08 92
Fax: +90 212 629 98 89
info@archem.com.tr   www.archem.com.tr
info@validity.com.tr   www.validity.com.tr

CE

SYMBOLS

Symbol	Description
IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
R1	Reagent 1
GTIN	Global Trade Item Number
REF	Reference Number
GLP	Good Laboratory Practices
FOR USE WITH	Identifies Products to Be Used Together
PRODUCT OF TURKEY	Product of Turkey
	Manufacturer
	Expiration Date
	Temperature Limits
	Consult Instructions for Use
	Caution
	Number of Tests