Triglycerides – Lipoprotein-lipase
Instructions for use (IFU)

IVD CE

ORDERING INFORMATION

	Code	Composition
OPEN KONELAB INDIKO	REF B75182532	n° 6 flaconi x 60 mL
	REF B75182533	n° 12 flaconi x 20 mL
CHEMILAB	REF B81180161	n° 3 vials x 28 mL
	REF B81180162	n° 10 vials x 34 mL

INTENDED USE
Product for use in the quantitative determination in vitro of the Triglycerides concentration in human serum or plasma. The results of the test must always be interpreted in conjunction with the clinical context. FOR PROFESSIONAL USE ONLY.

CLINICAL SIGNIFICANCE
Triglycerides constitute 95% of the lipids reserve in the human organism and the predominant form of glycerol esters present in the plasma. Thanks to the action of lipases and biliary salts, triglycerides are hydrolyzed into glycerol and fatty acids, and transported in the plasma by the Apo lipoproteins. The lipoproteins with the highest percentage of triglycerides are the chylomicrons and the very low-density lipoproteins (VLDL). The combination of an increase in LDL Cholesterol and triglycerides is an aggravating factor in the risk of coronary disease. Hypertriglyceridemia is a common disease in adults and is associated with diseases such as diabetes mellitus, insulin-resistance or obesity. Secondary hypertriglyceridemia on the other hand is associated with much more serious conditions such as liver and renal disease, hyperthyroidism and pancreatitis.

PRINCIPLE OF THE METHOD
Method Lipoprotein-lipase (LPL). Glycerol released by the hydrolysis of triglycerides with lipoprotein-lipase (LPL), is transformed by glycerol-Kinase (GK) to glycerol-3-phosphate which in turn is oxidised by glycerol-phosphate-oxidase (GPO) to di-hydroxyacetone-phosphate with the formation of hydrogen peroxide; this, in turn, in the presence of peroxidase (POD), reacts with ethyl-sulfopropyl-toluidine (ESPT) and 4-aminophenazone, giving rise to a coloured compound, the intensity of which is directly proportional to the triglyceride concentration in the sample.

$$\text{Triglycerides} \xrightarrow{\text{LPL}} \text{Glycerol} + \text{Fatty Acid s}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{Glycerol} - 3 - \text{phospate} + \text{ADP}$$

$$\text{Glycerol} - 3 - \text{phospate} + O_2 \xrightarrow{\text{GPO}} \text{Di} - \text{hidroxyacdate} - \text{phospate} + H_2O_2$$

$$2H_2O_2 + 4 - \text{aminophenazone} + \text{ESPT} \xrightarrow{\text{POD}} \text{Colouredcompound}$$

Storage and stability
= storage temperature 2-8°C
stored at 2-8 ° C avoiding direct light, the reagents are stable until the expiration date printed on the label.

Concentrations

Reagent:		
	Conc.	U.M.
PIPES buffer (pH 6.7)	20.0	mM
Adenosine-triphosfate (ATP)	1.00	mM
Lipoprotein-lipase (LPL)	350	KU/L
Magnesium ions	0.60	mM
Glicerol-chinase (GK)	40.0	U/L
Glycerol-3 phosphate-oxidase (GPO)	4000	U/L
Sodium Azide (NaN3)	14.6	mM
Ethyl-sulfopropyl-toluidine (ESPT)	2.00	mM
4-Aminophenazone	0.80	mM
Peroxidase (POD)	800	U/L

*Warning: The reagent is not classified according to CLP regulation

Reagents included in the kit
The reagent is described above.

Materials required but not supplied in the kit
Controls, calibrators and pipettes with adequate volume.

PRECAUTIONS and WARNINGS
1. Reagents and waste materials shall be disposed of in accordance with Community waste provisions or national or regional provisions.
2. Reagents may contain non-active components such as preservatives and detergents. The total concentration of these components is below the limits set out in Regulation 1272/2008 EC and subsequent amendments and additions.
3. It is recommended that the reagent be handled in accordance with the rules of good laboratory practice and that appropriate personal protective equipment be used.
4. Do not use the reagent if it is visibly degraded (e.g. presence of corpuscles).
5.All human samples shall be handled and disposed of as potentially infectious material.
6. The kit should only be used by qualified and properly trained technical personnel.
7. Diagnoses shall be carried out exclusively by authorised and qualified personnel.
8. Comply with national directives on occupational safety and quality assurance.
9. Use equipment that complies with current regulations.

Reporting of serious incidents
In the event of a serious incident in relation to the use of the device, please inform the manufacturer (via your distributor) and the competent authority of the European Union member state where the incident occurred. For other jurisdictions, reporting must be made in accordance with regulatory requirements. Reporting serious incidents helps provide more information about the safety of the diagnostic medical device.

PROCEDURE
Quality control
Control sera with a known titer of Triglycerides are commercially available for quality control, with values and confidence limits included. Sclavo Diagnostics Normal and pathological control sera are available: Clinicontrol N 5x5mL cod. B35181700 and Clinicontrol A 5x5 mL code B35181701. The values obtained must be within the acceptability range.

Calibration
For calibration use the “Calibrator serum Sclavo” Code B35181702.

Traceability
The Triglycerides traceability is reported in the package insert supplied with the calibrator serum.

SAMPLE
Type of sample and storage
Serum or plasma samples collected with EDTA or heparin should be used. Samples can be stored for 7 days at 4-8°C or 3 months at – 20°C.

PREPARATION OF THE REAGENT
The reagent is liquid, ready for use. After opening, the reagent is stable for 30 days if closed and stored at 2-8°C protected from direct light. Do not mix different batches. Slight variations in colour from batch to batch, will not affect test results.

Automation
The kit can be used with all automatic analyzers that can meet the operating conditions of the reagent while maintaining the volumetric ratios R /C. Validated applications are available for Sclavo Konelab® - Indiko® and CHEMILAB instruments. Applications not approved by Sclavo Diagnostics do not guarantee the performance of the reagent and must therefore be approved under the responsibility of the user.

MANUAL METHOD
The kit, in Open format, can be used manually through the use of spectrophotometer or photometer with the following parameters:

Reaction conditions

Wavelength (primary):	550 nm
Temperature:	37°C
Reaction	End point (Increasing reaction)

Technical
- Bring the reagents to reaction temperature and operate away from direct light.

	U.M.	Blank	Calib. Serum	Sample
Reagent	µL	1000	1000	1000
Calib. Serum	µL	-	10	-
Sample	µL	-	-	10
Blank	µL	10	-	-

Mix well and let stand for 10 minutes at 37°C before reading.
Measure absorbance of the sample and calibrator serum against reagent blank.
The reaction volumes may be varied proportionally without alteration of results.

Results:
Manual Method
Calculation of Triglycerides concentration:

$$\frac{\text{O.D. Sample}}{\text{O.D. Calibrator Serum}} \times \text{Concentr.Calibrator serum} = \text{Triglycerides mg/dL}$$

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Automation
The results are automatically calculated by the analyzer based on the calibration line. The analyzer automatically performs calibration in accordance with the method protocol. The calibration line is calculated automatically by the different instruments.

REFERENCE RANGE
Serum or plasma:
Male and Female:
- Normal values: < 150 mg/dL (<1,7 mmol/L)
- Borderline values: 150 - 199 mg/dL (1,7 – 2,25 mmol/L)
- High values: 200 - 499 mg/dL (2,26 – 5,64 mmol/L)

Each laboratory must establish its own normal values on the basis of its local population.

ANALYTICAL CHARACTERISTICS / PERFORMANCE
Linearity
The reaction is linear up to 1330 mg/dL (15.01 mmol/L). If the value in the sample exceeds the linearity limit of the method, dilute the sample with saline and multiply the result for the dilution factor.

Recovery
Commercial control sera were analyzed with the Triglycerides kit following the guidelines of the CLSI protocol. The data obtained are shown in the table below.

Range	Replicates	Mean	DS	CV%	Recovery
Low	5	83.6	1.14	1.36	97.4 %
High	5	189	2.38	1.26	90.1 %

Interferences
The high dilution of the sample with the reagent minimizes interference due to lipids.

Interference	Limits
Bilirubin	**30 mg/dL**
Haemoglobin	**500 mg/dL**

Precision of the method

Within-run precision					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	mg/dL	86.0	0.96	1.12	30
High	mg/dL	192	3.44	1.78	30
Between-run precision					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	mg/dL	83.0	1.70	2.05	20
High	mg/dL	199	3.93	1.98	20

Limit of Sensitivity

The Sensitivity limit has been measured using serial dilutions of high concentrated sera. The smallest detectable concentration is of about 3.2 mg/dL of Triglycerides in the conditions established for this test.

Comparison between methods
The Sclavo method for Triglycerides was compared with a similar commercially available method, analyzing 200 human samples. The correlation data between the two methods are reported in the table below.

Parameter	Estimation
Intercept	10.890
Slope	0.86
Correlation Coeff. (R)	0.996

Symbols used in IFU and Packaging	
IVD In vitro diagnostic medical device vitro	Manufacturer
REF Catalogue Number	Instruction for use
LOT Lot Number	Temperature limitation
Expiration date	

REFERENCE

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REVISION	DATE	CHANGE
Rev.A	01/2023	New Issue for IVDR Regulation (UE) 2017/746 compliance

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