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AZIENDA CERTIFICATA
UNI EN ISO 9001:2008
UNI EN ISO 13485:2004
CERTIFIED COMPANY

CHOLESTEROL SL

Enzymatic colorimetric method (CHOD-POD)

IVD CE In conformity with Directive 98/79 CE

000271 Rev.5 of 06/04/10

REF		
	ASR01101	**4 x 100 ml + STD**
	ASKIT0802	**6 x 45 ml**

• Principle

Under the action of Cholesterol esterase (CHE), cholesterol esters split up in cholesterol and fatty acids.
Cholesterol Oxidase(CHOD) oxidizes the above mentioned cholesterol along with the free cholesterol then releasing Cholesterol-3-one and hydrogen peroxide.
In presence of Peroxidase (POD), the released hydrogen peroxide reacts with a phenol substitute and 4-aminoantipyrine to form a red dye compound.
The intensity of the red colour produced is directly proportional to the total cholesterol in the sample.

• Sample

Not hemolized fresh serum or plasma.
Note: Cholesterol in serum or plasma is reported stable for one week at room temperature (15 – 25°C), and approximately 6 months when stored in the refrigerator at – 20°C and protected against evaporation.
Shake and bring the samples at room temperature before using.

• Expected value

Serum, plasma		
Recommended intervals	Suspect values	High values
< 200 mg/dl	200 – 240 mg/dl	> 240 mg/dl

Consider the above mentioned values as a reference.
It is strongly recommended that each laboratory establishes its own normal range according to its geographic area.

• Kit composition and possible risk classification

REAGENT (A)	
Good's Buffer	100 mmol/l
Cholesterol esterase	< 500 U/l
Cholesterol oxidase	< 500 U/l
Peroxidase	< 2000 U/l
4-AAP	1 mmol/l
Phenol derivates	5 mmol/l
Standard (B)	
Cholesterol	200 mg/dl

Avoid pipetting with mouth.
The kit doesn't contain substance or prepared classified as dangerous according to the currently legislation.

• Package: collection and storage

Store in refrigerator (2 – 8°C). Do not freeze.
Stable until the expiration date reported on the package.
After unsealing, it is advised to close up the bottle immediately in order to avoid evaporation, direct light exposure and bacteric contamination.

• Reagent preparation and stability

Liquid reagent ready to use, must be at room temperature (15 – 25°C) before using.
The Reagent is limpid and slightly pink coloured.
Stable until the expiration date reported upon the package.
The reagent slight colour (less than 0.030 O.D.) due to air or direct light exposure, will not affect its performances.

• Precautions and warning

Whenever agents infettantis, chemical reagents, reagents of human/animal origin, blood or other biological liquids are manipulated, it is advisable to follow the most common recommendations and take all the necessary hygienic precautions as the monouse gloves.

• Waste disposal

Please consult local regulations for a correct waste disposal.

• Operative method

Wavelength:	510nm (500-520)
Cuvette:	1 cm
Temperature:	+37°C
Zero operation:	Against blank reagent
Assay type:	End Point
Sample/Reagent ratio:	1/100

Pipetting in tubes:	*Blank*	*Sample*	*Standard*
Reagent	1000 µl	1000 µl	1000 µl
Distilled water	10 µl		
Sample		10 µl	
Standard			10 µl

Mix, incubate for 5' at 37°C and read sample and standard extinction.
Colour is stable at least 15' at room temperature.
Volumes can be proportionally modified.
This methodology describes the manual procedure to use the kit.
Calibration with watery standard may cause a systematic error when using automatic instrumentations.
Human proteic calibrator REF. ASR02031 is suggested.

• Calculation

$$\textbf{Cholesterol}\ \text{mg/dl} = \frac{\text{(E) Sample}}{\text{(E) Standard}} \times 200\ \text{(standard value)}$$

Standard 200mg/dl = 5.17mmol/l.

• Performance

A. METHOD PERFORMANCE

Measure interval/linearity:	7 – 700 mg/dl
Detection limit (2DS):	7.02 mg/dl
Sensitivity:	1 mg/dl = 0.00179A (510nm)

B. INTRA-ASSAY PRECISION: n=30

	Mean	C.V.(%)
Low control	M = 96.43 mg/dl	2.36 %
Medium control	M = 186.93 mg/dl	1.86 %
High control	M = 313.31 mg/dl	1.52 %

C. INTER-ASSAY PRECISION: n=30

	Mean	C.V.(%)
Low control	M = 97.72 mg/dl	1.32 %
Medium control	M = 192.17 mg/dl	2.76 %
High control	M = 317.67 mg/dl	1.38 %

D. CORRELATION BETWEEN METHODS

This method compared with a correspondent one from the competition, has given the following results:
N = 40 r= 0.999 y = 1.02 x + 0.8

E. INTERFERENCE (in accordance with recommendations SFBC)

1. Bilirubine does not interfere up to 20 mg/dl
2. Hemoglobin does not interfere up to 500 mg/dl
3. Triglicerides don't interfere up to 1500 mg/dl

For a thorough evaluation of the interfering substances, consult: Young, D.S.,et al.,Clin.Chem. 21:1D (1975).

• Limitations

For concentration higher than 700 mg/dl, repeat the measure on sample diluted 1:2 with saline solution and multiply the results by 2.
Cholesterol oxidase is not solely specific for cholesterol.
It can oxidize analogous substances such as diidrocholesterol, 7–diedro cholesterol, 20-idrossicholesterol etc.
These substances are not normally present in a relevant percentage in serum.

• Quality Control

It is recommended to execute the quality control at every kit utilization to verify that values are within the reference range indicated by the methodology.
For this purpose the use of test serum REF. ASR02010 (Normal level) and REF. ASR02020 (Pathologic level) is suggested.

• Bibliography

Trinder P., Ann.Clin.Biochem. 6,24, (1969).
Vassault,A. et al. Ann.Biol.Clin.,44,686,(1986).
Allain C.C and al.,C.Chem.,20,470,(1974).