ANA BIO ISP CHOLESTEROL

(CHOD-POD Method)

For Miura Instruments

Intended Use
Cholesterol is a reagent kit used for the determination of total cholesterol based on enzymatic method using cholesterol esterase, cholesterol oxidase and peroxidase.

Principle
Cholesterol esterase (CHE) hydrolyses cholesterol esters into free cholesterol and fatty acids. In the second reaction cholesterol oxidase (CHO) converts cholesterol to cholest-4-en-3-one and hydrogen peroxide. In presence of peroxidase (POD), hydrogen peroxide oxidatively couples with 4-aminoantipyrine and phenol to produce red quinoneimine dye. The intensity of the red colour is proportional to the amount of total cholesterol present in the specimen.

$$\text{Cholesterol esters} + H_2O \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{Fatty Acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHO}} \text{Cholest-4-en-3-one} + H_2O_2$$

$$H_2O_2 + \text{Phenol} + \text{4 - AAP} \xrightarrow{\text{POD}} \text{Red Quinonimine dye} + H_2O$$

Components & Concentration of Reagents

Reagent	Component	Concentration
Enzyme Reagent	Goods Buffer PH 6.8	100 mmol/L
	Phenol	10 mmol/L
	4-AAP	0.5 mmol/L
	CE	≤300 U/L
	COD	≤100 U/L
	POD	≤500 U/L
	Stabilizers, excipients & surface active agents	

Reagent storage and stability
The kit should be stored at 2° - 8℃ and is stable till the expiry date indicated on the label. **DO NOT FREEZE THE REAGENT.**

Reagent Preparation
Liquid reagent ready for use. After opening the reagent is stable for 30 days if closed, stored at 2° - 8℃, and protect from direct light. Do not mix different batches.

Specimen collection and preservation
Blood should be collected in a clean and dry container. Fasting blood is preferred for cholesterol assay. Cholesterol in the serum is stable for 7 days when stored at 2°-8℃ and 60 days when stored at -20℃

Automation
This kit, though developed and manufactured to be used as manual assay and with I.S.E. Miura Analyzer, can be used also with other analyzers able to meet the specifications indicated in section "Reaction conditions – Test procedure" Application sheets are available for automatic instruments.
All applications not explicitly approved by KDPL. Cannot be guaranteed in terms of performance, and must there be established by the operator.

Calibration
For Calibration use the "Multicalibrator"

Calibration Stability
For the instrumentation series Miura, the calibration is recommended to be done every 10 days.

Materials required but not supplied in the kit
Calibrators and controls

Assay guidelines for Analyzer I.S.E. Miura

Analyte Name	Cholesterol	
Method Code	CHO	
Type	End-Point	
Unit	mg/dl	
Filter F1	505 nm	
Blank in	Use	
Step	Reaction Volume	U.M.
Volume reagent R1	200	µl
Sample Volume	2	µl
Final Incubation	300	Sec.

Normal range
1. Guidance value for Children : 90 - 160 mg/dl
2. Guidance value for Adult : 130 - 200 mg/dl

Note: Expected range varies from population to population and each laboratory should establish its own normal range.

Limitation
Reaction is linear up to 1000 mg/dl. If the cholesterol value exceeds 1000 mg/dl, then dilute the specimen suitability with normal saline and repeat the assay. In such case the results obtained should be multiplied by dilution factor to obtain correct cholesterol value.

Quality Control
To ensure adequate quality control measures, it is recommended that each batch should include a normal and an abnormal commercial reference control serum. It should be realized that the use of quality control material checks both instrument and reagent functions together. Factors which might affect the performance of this test include proper instrument function, temperature control, cleanliness of glassware, Wavelength setting, Expiration date of reagents and accuracy of prob aspiration.

Accuracy-Recovery
Cholesterol added to a serum matrix containing known amounts of Cholesterol gave an average recovery of 96%.

Interference
The high dilution of the sample with the reagent reduces to a minimum the interference by lipids. Bilirubin below 5.8 mg/dl does not interfere in the reaction. Haemoglobin interferes at concentrations above 10.0 g/L.

Precision of the Method

Within-run					
Range	U.M	Mean	S.D.	C.V. (%)	No. run
Low	mg/dl	129.75	3.34	2.57	20
High	mg/dl	205.85	3.50	1.70	20
Between run					
Range	U.M	Mean	S.D.	C.V. (%)	No. run
Low	mg/dl	130.19	3.47	2.67	60
High	mg/dl	204.45	3.71	1.82	60

Sensitivity
At 505 nm a concentration of 7.0 mg/dl of Cholesterol can estimate.

References

1. Richmond,W.Clin. Chem. 19, 1350 (1973).
2. Allain C.C et al, Clin. Chem., 20, 470 (1974).
3. Tarbutton P.N., Gunter C.R., Clin. Chem, 20,724(1974).
4. Richmond,W. scan. J. Clin. Lab. Invest. 29, Suppl. 26, Abst. 3.25 (1972).
5. Young D.S. et al, 21, D (1975).

Symbols

IVD	In Vitro Diagnostics		Caution
LOT	Batch No.		Product Expiry Date
CONT	Content		Manufactured By
	Read Instructions		Date of Manufacture
	Storage Temperature		Keep Dry
REF	Catalogue No.		Fragile
			Keep away from sun light

KEE DIAGNOSTICS PVT LTD
(Formerly known as Kee GAD Biogen Pvt. Ltd.) .
CIN: U24231DL2004PTC128343 .
A subsidiary of KEE PHARMA LTD
A-8 . 3RD FLOOR. NARAINA INDUSTRIAL AREA, PHASE 2 . NEW DELHI 110028.
W. www.keediagnostics.in.
E. info@keediagnostics.in, T. 011 43136000.