ANA BIO CLR CHOLESTEROL

(CHOD-POD Method)

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 which has absorbance maximum at 510 nm (500 – 530 nm). The intensity of the red colour is proportional to the amount of total cholesterol present in the specimen.

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

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

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

Reagents provided

1. Cholesterol enzyme reagent - Ready to use.
2. Standard - Cholesterol (200 mg/dl).

Reagent storage and stability

The kit should be stored at 2° - 8°C and is stable till the expiry date indicated on the label. DO NOT FREEZE THE REAGENT. The reagent should be stored only in the amber bottle provided to protect it from direct light. Before use, swirl the reagents gently. DO NOT SHAKE VIGOROUSLY. Over the period of storage, the reagent may develop a light pink colour. This is expected and does not affect the reagent performance. Discard the reagent if the absorbance of the same exceeds 0.300 OD against distilled water blank at 510 nm. Contamination of the reagent should be strictly avoided. If the reagent develops turbidity discard the reagent.

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°C and 60 days when stored at -20°C

Assay guidelines for Analyzers

Reaction type	End point with standard
Reaction slope	Increasing
Incubation time	5 min. at 37°C / 10 min. at RT (25° - 30°C)
Wavelength	510 nm (500 - 530 nm)
Blank	Reagent Blank
Blank absorbance limit	< 0.300 Abs. against distilled water blank
Sample volume	10 µl (0.01 ml)
Reagent Volume	1000 µl (1.0 ml)
Standard Concentration	200 mg/dl
Factor Calculation	200 mg/dl ÷ Absorbance of Standard
Low Normal	130 mg/dl
High Normal	200 mg/dl
Linearity	Up to 1000 mg/dl

Assay guidelines for Manual procedure

Bring the enzyme reagent and standard to room temperature before performing the assay.

Reagents	**Blank**	**Standard**	**Sample**
Enzyme reagent	1000 µl (1.0 ml)	1000 µl (1.0 ml)	1000 µl (1.0 ml)
Standard	-	10 µl (0.01 ml)	-
Sample	-	-	10 µl (0.01 ml)

1. Mix thoroughly and incubate at 37℃ for 5 minutes or 10 minutes at room temperature (25° - 30℃).
2. Read the absorbance against reagent blank at 510 nm (500 - 530 nm).
3. The final colour is stable for 2 hours if not exposed to direct light.

Calculation

$$\text{Con.in sample (mg/dl)} = \frac{\text{Sample OD.}}{\text{Std.OD.}} \times \text{Con. of Standard}$$

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.
- The standard is a viscous solution. Use broad mouth pipette for accurate pipetting.

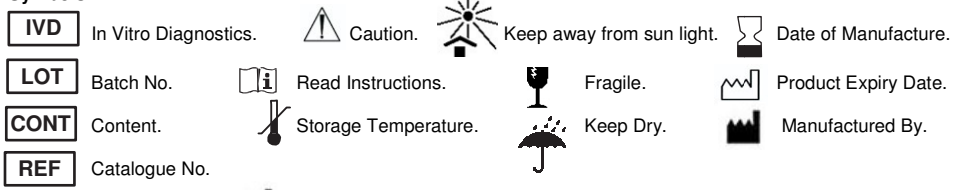
Quality Control

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 and accuracy of pipetting.

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



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(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.