INTRODUCTION

1. *Infinite* Liquid Cholesterol is a reagent set for determination of total cholesterol based on **enzymatic method** using Cholesterol esterase, Cholesterol oxidase and Peroxidase.
2. *Infinite* Liquid Cholesterol is a **ready-to-use reagent.**
3. *Infinite* Cholesterol can be determined in ust **5 minutes** at 37°C or **10 minutes** at R.T. (25-30°C).
4. *Infinite* Liquid Cholesterol is **linear** upto 1000 mg%.
5. *Infinite* Liquid Cholesterol can be used on any **Colorimeter Spectrophotometer, Discrete semiautomated and Automated analyzer.** Programme can be designed for any specific analyzer upon request.
6. The influence of **Ascorbic acid, Bilirubin, Haemoglobin, Glucose, Sodium fluoride, Heparin, EDTA, Creatinine and Uric acid** is negligible.
7. *Infinite* Liquid Cholesterol reagent system can also be used for determining HDL Cholesterol. The **HDL ppt reagent and standard** can be ordered separately.

PRINCIPLE

Cholesterol esterase hydrolyses cholesterol esters into free cholesterol and fatty acids. In the second reaction cholesterol oxidase converts cholesterol to cholest-4-en-3-one and hydrogen peroxide. In presence of peroxidase, hydrogen peroxide oxidatively couples with 4 - aminoantipyrine and phenol to produce red quinoneimine dye which has absorbance maximum at 510 nm. (500 - 530). The intensity of the red colour is proportional to the amount of total cholesterol in the specimen.

Cholesterol esters $\xrightarrow{\text{CHE*}}$ **Cholesterol + Fatty acids**

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

$2H_2O_2$ + 4-aminoantipyrine + Phenol $\xrightarrow{\text{POD*}}$ **Red Quinoneimine Dye + H_2O**

*Abbreviations

CHE	=	Cholesterol esterase
CHO	=	Cholesterol oxidase
POD	=	Peroxidase

REAGENT STORAGE & STABILITY & HANDLING

The kit should be stored at 2- 8°C and is stable till the expiry date indicated on the label.

The reagent and standard are ready-to-use and are stable till expiry, when stored at 2- 8°C. **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 in the reagent gently. **DO NOT SHAKE VIGOROUSLY.**

Over time, 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 O.D. against distilled water at 510 nm.

Contamination of the reagent should be strictly avoided. Should the reagent develop turbidity discard the reagent.

COMPONENTS & CONCENTRATION OF WORKING SOLUTION

Component	Concentration
• Buffer, pH 6.8	50 mmol/l
• Cholesterol oxidase	≥ 100 IU/l
• Cholesterol esterase	≥ 150 IU/l
• Peroxidase	≥ 500 IU/l
• 4 - aminoantipyrine	0.5 mmol/l
• Phenol	≥ 10 mmol/l
• Stabilizers / Surfactants	

SPECIMEN COLLECTION & PRESERVATION

Blood should be collected in a clean 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.

PROCEDURE

- ☐ **Reaction type** ..... **End-Point**
- ☐ **Reaction time** ..... **5 mins. at 37°C/10 mins. at R.T. (25-30°C)**
- ☐ **Wavelength** ..... **510 nm. (500 - 530 nm.)**
- ☐ **Zero setting with** ..... **Reagent Blank**
- ☐ **Blank absorbance limit** ..... **≤ 0.300 Abs.**
- ☐ **Sample volume** ..... **0.01 ml (10 µl)**
- ☐ **Reagent volume** ..... **1.0 ml**
- ☐ **Standard concentration** ..... **200 mg%**
- ☐ **Linearity** ..... **1000 mg/dl**

Manual assay procedure

Prewarm at room temperature the required amount of reagent before use.

Perform the assay as given below :

1.0 ml procedure

	Serum	Standard	Blank
	0.01 ml	0.01 ml	—
Reagent	1.0 ml	1.0 ml	1.0 ml

Incubation

Incubate the assay mixture for 5 minutes at 37°C or 10 minutes at room temperature (25-30°C). After incubation measure the absorbance of assay mixture against blank at 510 nm. The final colour is stable for two hours if not exposed to direct light.

Calculation:

❶ **With Standard**

$$\text{Conc. (mg\%)} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times 200$$

❷ **With factor for wavelength range : 500 - 510 nm.**

Conc. (mg%) = 543 x Absorbance of sample

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NOTE :

1. The specimen to working reagent ratio can be altered proportionally without affecting the results.
2. Falsely elevated results are obtained due to lipemic samples. The ***Infinite*** Liquid Cholesterol overcomes falsely elevated result with use of Lipid clearing agent in the reagent formulation. Lipid clearing agent clears up the turbidity caused by lipemic samples.

EXPECTED VALUES

Desirable Cholesterol	:	**< 200 mg/dl**
Borderline High Cholesterol	:	**200 - 239 mg/dl**
High Cholesterol	:	**> 240 mg/dl**

Expected range varies from population to population. It is recommended that each laboratory should establish its own normal range.

PROCEDURE LIMITATIONS

1. If the cholesterol value exceeds 1000 mg% then dilute the specimen suitably with normal saline & repeat the assay. In such case the results obtained should be multiplied by dilution factor to obtain correct cholesterol value.
2. The standard is a viscous solution. Use broad mouth pipette for accurate pipetting.

QUALITY CONTROL

To ensure adequate quality control, 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. Tarbutton, P.N., Gunter, C.R., ***Clin. Chem., 20, 724*** (1974).
3. Allain, C.C. et al, ***Clin Chem, 20, 470*** (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)

IVD	*In Vitro* Diagnostic Use		Date of Manufacturing
	Consult Instructions for use		Use by (YYYY-MM-DD)
REF	Catalogue Number		Temperature Limitation
LOT	Batch Code		Manufacturer

CE European Conformity

AR. No.: I 25 *LC-2009-03-00I*

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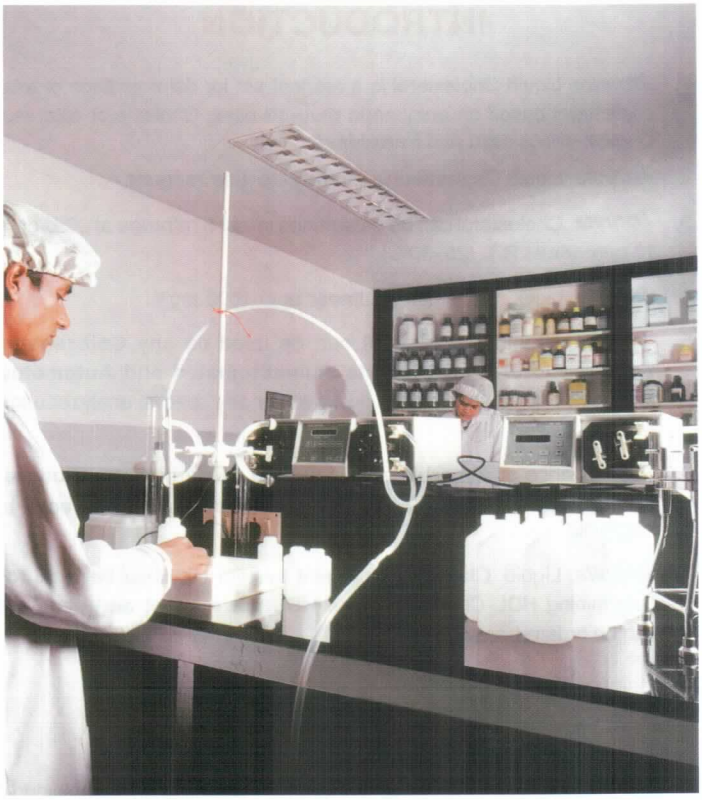
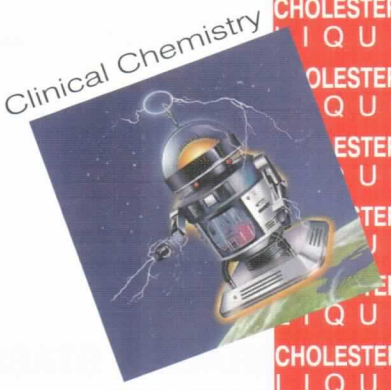


Liquid dispensing facility



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CHOLESTEROL

Liquid

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