

INTRODUCTION

- Infinite** Liquid Cholesterol is a reagent set for determination of total cholesterol based on **enzymatic method** using Cholesterol esterase, Cholesterol oxidase and Peroxidase.
- Infinite** Liquid Cholesterol is a **ready-to-use reagent**.
- Infinite** Cholesterol can be determined in **just 5 minutes** at 37°C or **10 minutes** at R.T. (25-30°C).
- Infinite** Liquid Cholesterol is **linear** upto 1000 mg%.
- 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.
- The influence of **Ascorbic acid, Bilirubin, Haemoglobin, Glucose, Sodium fluoride, Heparin, EDTA, Creatinine and Uric acid** is negligible.
- 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.



*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:

① With Standard

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

② With factor for wavelength range : 500 - 510 nm.

$$\text{Conc. (mg\%)} = 543 \times \text{Absorbance of sample}$$

