CONCEPT DIAGNOSTICS
ONTARIO, CALIFORNIA 91761 USA

CRESTLINE
SCIENTIFIC CORPORATION
2902 Finlandia cor Copernico Streets, San Isidro Village, Makati City
Telefax No.: 887-5777 / 887-2150

CHOLESTEROL REAGENT SET (PHENOL FREE)

CHOLESTEROL REAGENT SET (PHENOL FREE)
For the quantitative determination of total cholesterol in serum.

INTRODUCTION
Cholesterol is a fatty substance found in blood, bile and brain tissue; it serves as a precursor to bile acids, steroids and vitamin D. The determination of serum cholesterol is a major aid in the diagnosis and classification of lipemias.[1] Other conditions such as hepatic thyroid diseases influence cholesterol levels.[2]

Enzymatic methods have replaced older methodologies involving cholesterol esterase and oxidase and Trinders color system. Allain et al. developed a total enzymatic technic in which hydrogen peroxide during the oxidation of cholesterol is used in conjunction with peroxidase, 4-aminoantipyrine and phenol to form a quinoneimine dye.[3] This reagent employs p-hydroxy benzene sulfonic acid (p-HBS), in place of phenol to produce a quinoneimine dye with greater absorbance at 520nm and a surfactant to facilitate the completion of reaction.

PRINCIPLE
The enzymatic reaction sequence employed in the assay of cholesterol is as follows:

Cholesterol Esters $\xrightarrow{\text{C. Esterase}}$ Cholesterol + Fatty Acids

Cholesterol + 0_2 $\xrightarrow{\text{C. Oxidase}}$ Cholesten-3-one + H_20_2

2 H_20_2 + 4-Aminoantipyrine

+ p-HBS $\xrightarrow{\text{H.Peroxidase}}$ Quinoneimine + 2 H_20
(red dye)

Cholesterol esters are hydrolyzed to produce cholesterol. Hydrogen peroxide is then produced from the oxidation of cholesterol by cholesterol oxidase. In a coupled reaction catalyzed by peroxidase, quinoneimine dye colored red is formed from 4-aminoantipyrine, p-HBS and hydrogen peroxide. The absorption at 520nm of the solution of this dye is proportional to the concentration of cholesterol in the sample.

REAGENT COMPOSITION
When reconstituted as directed, the reagent for Cholesterol contains the following:

1. CHOLESTEROL REAGENT:
   (Concentrations refer to the reconstituted reagent.) 4-Aminoantipyrine 0.6mM, Sodium Cholate 8.0mM, Cholesterol Esterase > 150 U/L, Cholesterol Oxidase > 200U/L, Horseradish Peroxidase > 1500U/L, p-Hydroxy benzene sulfonate 20mM, Buffer 125mM, pH 6.8, non reactive stabilizers and fillers.
2. CHOLESTEROL STANDARD:
   200mg/dl cholesterol in alcohol.
   Store at 2 - 8° C and keep tightly capped.

WARNINGS AND PRECAUTIONS
1. For in vitro diagnostic use.
   CAUTION: Do not pipette reconstituted reagent by mouth as the effects are unknown.
2. Specimens should be considered infectious and handled appropriately.
3. Use distilled or deionized water where indicated.

STORAGE AND STABILITY
Store the reagent set at 2 - 8° C. Store the reconstituted reagent at 2 - 8° C (refrigerated). The reconstituted reagent is stable for sixty (60) days when stored in an amber bottle at 2 - 8°C.[4]

REAGENT DETERIORATION
The reagent should be discarded if:
1. Turbidity has occurred; turbidity may be a sign of contamination.
2. Moisture has penetrated the vial and caking has occurred.
3. The reagent fails to meet linearity claims or fails to recover control values in the stated range.

SPECIMEN COLLECTION
1. Test specimens should be serum and free from hemolysis.
2. Cholesterol in serum is reported stable for seven (7) days at room temperature (18 - 25°C) and six (6) months when frozen and properly protected against evaporation.

INTERFERING SUBSTANCES
Anticoagulants such as fluoride and oxalate will result in false low values.[5] The test is not influenced by hemoglobin values up to 200mg/dl or by bilirubin levels up to 10mg/dl. Interference from grossly icteric and heavily hemolyzed specimens is correctible by use of a serum/plasma blank.

MATERIALS REQUIRED BUT NOT PROVIDED
1. Spectrophotometer capable of measuring absorbances at 520nm.
2. Test tubes/rack.
3. Accurate pipetting/measuring devices.
4. Timer.
5. Heating block (37° C).

GENERAL INSTRUCTIONS
The reagent for Cholesterol is intended for use either as an automated procedure on chemistry instruments or as a manual procedure on a suitable spectrophotometer.

PROCEDURE (AUTOMATED)
Consult the appropriate instrument application guide available from us.

PROCEDURE (MANUAL)
1. Prepare reagent according to instructions on vial label.
2. Label test tubes: blank, standard, control, patient, etc.
3. Pipette 1.0ml of reagent to all tubes and pre-warm at 37°C for at least two (2) minutes.

4. Add 0.01ml (10µl) of sample to respective tubes, mix and return to 37°C

5. Incubate all tubes at 37°C for ten (10) minutes.
6. Zero spectrophotometer with the reagent blank at 520nm. (Wavelength range:500-550).
7. Read and record absorbances of all tubes.

* USE MULTI PURPOSE CALIBRATOR TO REPLACE STANDARD.

N0TE:

If the spectrophotometer being used require a final volume greater than l.0ml for accurate reading, use 0.025m1 (25µ1) of sample to 3.0 ml of reagent. Perform the test as described above.

PROCEDURAL LIMITATIONS

The reagent is linear to 500mg/dl.

1. Samples with values above 500mg/dl should be diluted 1:1 with isotonic saline and re-run. Multiply final results by two (2).
2. Grossly lipemic serums require a "sample blank." Add 0.02ml (20µl) of sample to 2.5ml saline, mix and read the absorbance against water. Subtract this value from the patient absorbance to obtain the corrected reading.

CALCULATIONS

(A= Absorbance)

$$\frac{A\ (patient)}{A\ (standard)} \times \text{Concentration of standard. (mg/dl)} = \text{Concentration of patient (mg/dl)}$$

Example:
A (patient) = 0.40, A (standard) = 0.32,
Concentration of standard = 200mg/dl.

$$\frac{0.40}{0.32} \times 200 = 250 \text{mg/dl}$$

QUALITY CONTROL

It is recommended that controls be included in each set of assays. Commercially available control material with established cholesterol values may be used for quality control. The assigned value of the control material must be confirmed by the chosen application. Failure to obtain the proper range of values in the assay of control material may indicate either reagent deterioration, instrument malfunction, or procedural errors.

EXPECTED VALUES[6]

It is strongly recommended that each laboratory establish its own normal range.

RISK CLASSIFICATION	TOTAL CHOLESTEROL IN BLOOD (mg/dl)
Desirable	< 200
Borderline	200-239
high	≥ 240

PERFORMANCE CHARACTERISTICS

1. Linearity: 500mg/dl.
2. Comparison: A comparison between this procedure and one utilizing phenol free produced a regression equation of Y = 0.95X + 10.3 with a coefficient of correlation of 0.98.
3. Precision:

Within Run

Mean (mg/dl)	S.D.	C.V.(%)
127	3.6	2.8
330	4.9	1.4

Run to Run

Mean (mg/dl)	S.D.	C.V.(%)
130	4.7	3.6
324	8.2	2.5

4. Specificity: Cholesterol Oxidase is not totally specific for cholesterol. Other analogs of cholesterol (dihydrocholesterol, 7-dehydrocholesterol, 20 hydroxycholesterol, etc.) are also oxidized. These analogs do not normally occur in any appreciable amounts in serum.

REFERENCES

1. Beaumont, J.L., Crison, L.A., Cooper, G.R., Feifar, Z., Frederickson, D.S., and Strasser, T.; "Classification of Hyperlipidemias and Hyperlipoproteinemias." *Standard Methods of Clinical Chemistry* vol. 9, Academic Press, New York, NY (1972).
2. Holvey, D.N., ed. *The Merck Manual of Diagnosis and Therapy*. Merck and Co., Inc. Rahyway, NJ (1972).
3. Aliain, C.C., et. al., *Clin. Chem.* 20:470 (1974).
4. Perlstern, M.T., et al., *Micro. Chem. J*. 22:403 (1977).
5. Young, D.S., et al., *Clin. Chem.* 21 No. 5 (1975).
6. Naito, H.K., et. al., *Clin. Chem.* 34:193 (1988).

Date Revised: 07/96