

Cholesterol Reagent

Catalog #: 43710

for use with the

SDI CA480 Clinical Chemistry System

INTENDED USE

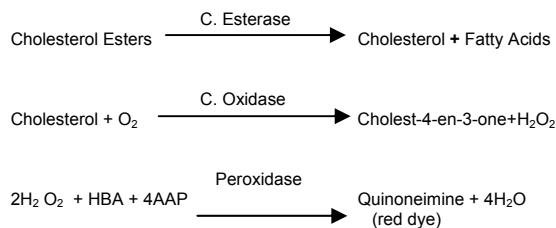
For the *in vitro* quantitative determination of Total Cholesterol in serum.

METHODOLOGY

A Cholesterol method developed in the late 1800's by Lieberman¹ and Burchard² is still in use today despite its corrosive nature and its susceptibility to many interfering substances.

Work on an enzymatic procedure was begun by Flegg³ and Richmond⁴ in the early 70's. Allain⁵ and Roeschlau⁶ began using cholesterol esterase and oxidase, in a single reagent to determine total cholesterol in serum. Trinder's⁷ color system of peroxidase / phenol /4-aminoantipyrine has been used successfully for some time now. The system's only drawback was the corrosive properties of phenol. The present method utilizes a phenol substitute that performs like phenol but without being corrosive.

Principle:



The intensity of the red color produced is directly proportional to the total cholesterol in the sample when read at 500nm.

REAGENT COMPOSITION

Active Ingredients	Concentration
4-Aminoantipyrine	0.25 mM
Cholesterol Oxidase	400 U/L
Lipoprotein Lipase	300 U/L
Horseradish Peroxidase	1000 U/L
HBA	10 mM
Surfactant	
Buffer	
pH 6.75	
Sodium Azide (.01%) as a preservative.	

Precautions

1. This reagent is for *in vitro* diagnostic use only.
2. Reagent contains sodium azide. Poison. Do not ingest. May react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with a large volume of water to prevent build up.

REAGENT PREPARATION

Reagent comes in a ready to use form.

REAGENT STORAGE

1. Store the reagent at 2-8°C (refrigerated).
2. The reagent is stable until the expiration date when stored at 2-8°C.

REAGENT DETERIORATION

The reagent should not be used if:

1. The reagent is turbid.
2. The working reagent does not meet stated performance parameters.

SPECIMEN COLLECTION AND STORAGE

1. Nonhemolyzed serum is recommended.
2. Cholesterol in serum is reported stable for seven days at room temperature (18-25°C) and six months when frozen and properly protected against evaporation.^{8,9}

INTERFERENCES

Studies to determine the level of interference for hemoglobin, bilirubin, and lipemia were carried out, the following results were obtained:

Hemoglobin:

No significant interference ($\pm 10\%$) from hemoglobin up to 200 mg/dL.

Bilirubin:

No significant interference ($\pm 10\%$) from bilirubin up to 19.8 mg/dL.

Lipemia:

No significant interference ($\pm 10\%$) from lipemia up to 1093 mg/dL measured as triglycerides.

A number of drugs and substances may affect the accuracy of this test. See Young, et al¹⁰

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED

1. SDI CA480 Clinical Chemistry System.
2. Deionized water and related equipment, e.g.: pipettes
3. Analyzer specific consumables, e.g.: sample cups
4. Control, and Calibrator materials such as those provided by SDI Biomed.

ASSAY PROCEDURE

System Parameters

Cholesterol (Liquid)	
TEMPERATURE:	37°C
WAVELENGTH:	500 nm
ASSAY TYPE:	Endpoint
DIRECTION:	Increase
SAMPLE / RGT RATIO:	1 : 100
e.g. Sample Vol.	0.01 mL (10µL)
Reagent Vol.	1.0 mL
INCUBATION TIME:	5 Min

Procedure Notes

1. The reagent and sample volumes may be altered proportionally to accommodate various instrument requirements.
2. Grossly lipemic serums require a "sample blank". Add 0.01m l (10ml) of sample to 1.0mL saline, mix and read the absorbance against water. Subtract this value from the patient absorbance to obtain the corrected reading.

Calculations

$$\begin{array}{l} \text{A (Absorbance)} \\ \text{A (patient)} \quad \times \quad \text{Concentration of standard} \quad = \quad \text{Cholesterol} \\ \text{A (standard)} \quad \quad \quad \text{(mg/dL)} \quad \quad \quad \quad \quad \quad \quad \text{(mg/dL)} \end{array}$$

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

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

Limitations

Samples with values exceeding 750 mg/dL should be diluted 1:1 with saline and re-run. The final answer should be multiplied by two.

CALIBRATION

Aqueous standards can be used to calibrate the procedure or an appropriate serum calibrator.

QUALITY CONTROL

The integrity of the reaction should be monitored by use of a two level control with known Cholesterol values.

EXPECTED VALUES¹¹

Recommended Range:

Desirable Cholesterol:	<200 mg/dL
Borderline-High Cholesterol:	200 – 239 mg/dL
High Cholesterol:	>240 mg/dL

PERFORMANCE

Linearity:

When run as recommended the assay is linear to 750 mg/dl

Method Comparison:

Studies performed between this procedure and a similar methodology yielded the following results:

Number of samples pairs:	49
Range of samples:	1 – 502 (mg/dL)
Correlation Coefficient:	0.9961
Slope:	1.015
Intercept:	-3.7(mg/dL)

Precision:

Within Run	Level 1	Level 2
Mean (mg/dL)	148	224
S.D. (mg/dL)	2.2	2.1
C.V. (%)	1.5	1.0

Total

	Level 1	Level 2
Mean (mg/dL)	148	224
S.D. (mg/dL)	3.5	6.3
C.V. (%)	2.3	2.8

Sensitivity:

A calibration factor of approximately 1702 was obtained, which is equivalent to a sensitivity of 0.0006 D Abs per mg/dL.

REFERENCES

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Manufactured for:



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