CHOLESTEROL (CHOD-PAP)

INTENDED USE
Bioline Cholesterol reagent is used for the quantitative determination of total cholesterol in serum.

CLINICAL SIGNIFICANCE
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 lipemia. Other conditions such as hepatic thyroid diseases influence cholesterol levels.

METHOD AND PRINCIPLE
Enzymatic methods have replaced older methodologies involving cholesterol esterase, oxidase, and Trinder's color system. Allain et al. developed a total enzymatic technique in which hydrogen peroxide during the oxidation of cholesterol is used in conjunction with peroxidase,4-aminoantipyrine and phenol to form a quinonimine dye. This reagent employs p-hydroxy benzene sulfonic acid (p-HBS),in place of phenol to produce a quinonimine dye with greater absorbance at 505 nm and a surfactant to facilitate the completion of reaction.
The results obtained from this replacement are equal to the results obtained based on the Abell-Kendall cholesterol reference method which is recommended by the CDC.

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

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

Cholesterol $+O_2$ $\xrightarrow{\text{C.Oxidase}}$ Cholesten-3-one$+H_2O_2$

$2H_2O_2$+ 4-Aminoantipyrine

+p-HBS $\xrightarrow{\text{H.Peroxidase}}$ Quinoneimine$+2H_2O$ (red dye)

Cholesterol estersare hydrolyzed to produce cholesterol. Hydrogen peroxide is then produced from the oxidation of cholesterol by cholesterol oxidase. In a coupled reaction catalyzed by peroxidase, quinonimine dye colored red is formed from 4-aminoantipyrine, phenol and hydrogen peroxide. The absorption at 505 nm of the solution of this dye is proportional to the concentration of cholesterol in the sample.

REAGENT COMPOSITION
Cholesterol (liquid) reagent set contains the following:
1. Cholesterol Reagent:
   4-Aminoantipyrine 0.6mM, Sodium Cholate 8.0mM, Cholesterol Esterase 150 U/L, Cholesterol Oxidase 150U/L, Horse-radishPeroxidase 1,200U/L, p-Hydroxy benzene sulfonate 20mM, Buffer125mM, pH6.8,non-reactive ingredients.
2. Cholesterol Standard :200 mg/dl.
   This standard was made with materials traceable to Standard Reference Material available from the National Institute of Standard and Technology.

WARNINGS AND PRECAUTIONS
For *invitro* diagnostic use.
CAUTION: Invitro diagnostic reagents may be hazardous. Handle in accordance with good laboratory procedures which dictate avoiding ingestion, and eye or skin contact.
Specimens should be considered infectious and handled appropriately.

REAGENT PREPARATION
All reagents come in a ready-to-use form. No preparation is necessary.

REAGENT STORAGE ANDSTABILITY
Reagent is stable till expiry , Store the reagent set at 2-8°C.

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

SPECIMEN COLLECTION AND STABILITY
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.

INTERFERENCES
Anti coagulants such as fluoride and oxalate will result in false low values. The test is not influenced by hemoglobin values up to 200mg/dl or by bilirubin levels up to 10 mg/dl. Interference from grossly icteric and heavily hemolyzed specimens is correctable by use of a serum blank.

ASSAY PROCEDURE FOR SEMIAUTO ANALYZER
Wavelength 505nm
Temperature 37ºC

	Blank	Standard	Sample
Reagent	1000 µL	1000 µL	1000 µL
Standard	-	10 µL	-
Sample	-	-	10 µL

Mix and read the optical density (OD) of standard and Sample against blank after 5 minutes of incubation at 37ºC.

CALCULATIONS
(A =Absorbance)

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

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

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

CALIBRATION
The procedures are calibrated with the standard solution, which is included with each series of tests. Its absorbance is used to calculate the results. It is recommended to establish a linearity curve up to 1000 mg/dl with other available commercial standard solutions to verify the performance of instruments and reagents

LIMITATIONS
The reagent is linear upto1000 mg/dl.
Samples with values above 1000 mg/dl should be diluted 1:1 with isotonic saline and re-run. Multiply final results by 2.
Grossly lipemic serums require a "sample blank."Add 0.01ml (10µl) of sample to 1.0 ml saline, mix and read the absorbance against water. Subtract this value from the patient absorbance to obtain the corrected reading.

QUALITY CONTROL

1. It is recommended that high and low values of cholesterol 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.
2. It is recommended that CDC or NIST certified reference material is included in the assay to assure the performance of the test.

EXPECTED VALUES

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

RISK CLASSIFICATION	TOTAL CHOLESTEROL (mg./dl)
Desirable	< 200
Borderline high	200-239
High	> 240

PERFORMANCE CHARACTERISTICS

1. ***Linearity*:**1000 mg/dl.
2. **Sensitivity**: An absorbance change of 0.001 at 505nm corresponds to 1 mg/dl under the stated condition of this assay system.
3. ***Comparison*:**A comparison between this procedure and one which is certified by the Center for Disease Control (CDC) and the National Cholesterol Education Program (NCEP) based on human samples assayed both by the Abell-Kendall's method and by the method being certified produced a regression equation of y=0.94x–2.80 (N=47)with a coefficient of correlation of 0.99.
4. **Precision:**

	WithinRun	
Mean(mg/dl)	S.D.	C.V.(%)
150	9.7	6.5
124	8.8	7.0
	Run-to-Run	
Mean(mg/dl)	S.D.	C.V.(%)
142	7.4	5.2
114	6.1	5.3

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

General Technical Parameters

Mode	**End Point**
Wavelength (Filter)	**505 nm**
Reaction Direction	**Increasing**
Reagent Blank	**Yes**
Sample Vol.	**10 µL**
Reagent Vol.	**1000 µL**
Incubation Time	**5 min**
Reagent Blank Abs (Max.)	**NMT 0.300 Abs**
Calibration Method	**1 - Point**
Standard (Conc.)	**200 mg/dL**
Linearity	**1000 mg/dL**
Decimal Places	**0**
Temp.	**37 °C**
Unit	**mg/dL**
Ref. Desirable (Male / Female)	**< 200 mg/dL**
Ref. Boderline high (Male / Female)	**200-239 mg/dL**
Ref. High (Male / Female)	**> 240 mg/dL**

REFERENCES

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