

# Cholesterol (Liquid)

## 1.0 INTENDED USE

This reagent is intended for the quantitative determination of total cholesterol in serum.

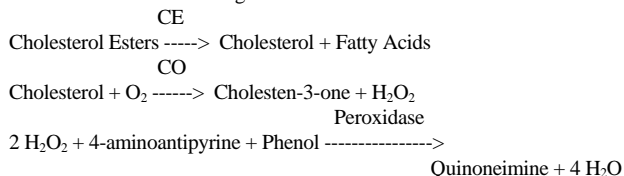
## 2.0 BACKGROUND

### 2.1 METHOD AND HISTORY

Cholesterol methodologies have been critically reviewed by Tonks (10.1) and more recently by Zak (10.2). The enzymatic method described below, and used in this kit, is a modification of that described in 1974 by Allain et. al.(10.3) and Roschlau et. al. (10.4).

### 2.2 TEST PRINCIPLE

The cholesterol esters are hydrolyzed to free cholesterol by cholesterol esterase (CE). The free cholesterol is then oxidized by cholesterol oxidase (CO) to cholesten-3-one with the simultaneous production of hydrogen peroxide. The hydrogen peroxide produced couples with 4-aminoantipyrine and phenol in the presence of peroxidase to yield a chromogen with maximum absorbance at 505 nm. This method is summarized in the following reactions:



The intensity of the color produced is directly proportional to the concentration of total cholesterol in the sample.

## 2.3 CLINICAL SIGNIFICANCE

Serum cholesterol is an important aid in the diagnosis and classification of hyperlipoproteinemias. Elevated levels of serum cholesterol are often predictive of an atherosclerotic process, the progress of which is monitored by observing changes in the level of serum cholesterol. Elevated levels may also be associated with hepatic, hypothyroid, and certain nephrotic disorders.

## 3.0 SPECIMEN COLLECTION AND HANDLING

### 3.1 PATIENT PREPARATION

Patient should be fasting for 12 hours prior to specimen collection.

### 3.2 SPECIMEN COLLECTION.

Fresh, clear, unhemolyzed serum is the preferred specimen. The specimen should be drawn in the morning following a 12-hour fast.

Use a standard venipuncture tube to draw patient sample.

The amount of sample required will depend on the analyzer used. The amount of serum required is in the range of 5-25  $\mu$ l. Call Biotron's technical service department at 1-800-595 8766 for the recommended sample volume for your analyzer.

Record the patient's name, date and time of sample collection and preparation.

## 3.3 SPECIMEN STORAGE

If the specimen is not assayed within 8 hours of collection, it is recommended that the specimen be stored under refrigeration in a tightly sealed container. Samples for cholesterol assay are stable for 1 week when refrigerated (2° to 8°C) and for 4 weeks when frozen (-20°C). Frozen samples should be thawed at room temperature and mixed thoroughly before analysis. Thawed samples should not be refrozen.

## 4.0 MATERIALS

(2 X 125 ml)  
(4 X 125 ml)

Reagents necessary for the determination of total cholesterol are included in the kit.

### 4.1 REAGENT

#### 4.1.1 Cholesterol Reagent contains:

peroxidase (horseradish)  $\geq 5500$  U/L  
cholesterol esterase (pancreas)  $\geq 300$  U/L  
cholesterol oxidase (nocardin)  $\geq 300$  U/L  
4-aminoantipyrine  $\geq 0.6$  mM  
phenol  $\geq 30$  mM

buffer, preservative, surfactants, and stabilizer

#### 4.1.2 Standard/Control/Calibrator

### 4.2 WARNINGS AND PRECAUTIONS

For In Vitro Diagnostic Use. Not for Internal use in Humans or Animals. In Vitro Diagnostics reagents may be hazardous. Avoid ingestion and skin or eye contact.

### 4.3 REAGENT PREPARATION

The reagent is ready to use as is.

### 4.4 REAGENT STORAGE AND STABILITY

When stored at 2°-8°C unopened reagents are stable until the expiration date printed on the label.

### 4.5 ADDITIONAL MATERIALS REQUIRED

4.5.1 Spectrophotometer or colorimeter capable of reading absorbance at 505 nm.

4.5.2 1 cm cuvettes or a flow cell capable of transmitting light at 505 nm.

4.5.3 Test tubes capable of holding 3 ml.

4.5.4 Pipettes capable of delivering 2.5 ml and 25  $\mu$ l.

4.5.5 Deionized or distilled water for preparing the reagent blank.

4.5.6 Timer for a 5 minute or 20 minute incubation.

4.5.7 Constant temperature source which can be adjusted to 37° C.

4.5.8 Calibrator

4.5.9 Normal and abnormal control for quality control.

## 5.0 TEST PROCEDURE

The following is a general procedure for use on a manual instrument.

### 5.1 PROCEDURE CONDITIONS

Wavelength 505 nm  
Temperature 37° C, or 18-26° C  
Pathlength 1.0 cm  
Mode endpoint  
Reaction time 5 min at 37° C  
20 min at 18-26° C

Sample volume 25  $\mu$ l  
Reagent volume 2.5 ml  
Total volume 2.525 ml  
Sample to reagent ratio 1/100

### 5.2 INSTRUMENT

Any instrument capable of reading absorbance accurately with a sensitivity of 0.001 absorbance at 505 nm may be used. The band width should be 10 nm or less, stray light 0.5% or less, and the wavelength accuracy within 2 nm.

### 5.3 CALIBRATION

5.4 The total cholesterol assay is calibrated by referencing the absorbance of the unknown sample to the absorbance of the calibrator.

## 5.4 PROCEDURE

- 5.4.1 Into separate test tubes pipette 25 µl of distilled water, cholesterol calibrator, or serum to be assayed.
- 5.4.2 Add 2.5 ml of cholesterol reagent and mix.
- 5.4.3 Incubate for 5 minutes at 37° C or 20 minutes at 18-26° C (room temperature) and determine the absorbance of the calibrator (As) and of each serum (A) at 505 nm using the distilled water sample as the reagent blank.

## 5.5 PROCEDURE NOTE

The final color is stable for 20 minutes.

## 5.6 CALCULATION AND RESULTS

$$\text{Total Cholesterol} = \frac{A}{A_s} \times \text{concentration of calibrator}$$

A = absorbance of sample  
As = absorbance of calibrator

Example:

$$\text{Total Cholesterol} = \frac{.485}{.352} \times 200 \text{ mg/dl} = 276 \text{ mg/dl}$$

with A = .485 and As = .352, concentration of calibrator = 200 mg/dl

Note: 1 mg/dl = .02587 mmol/L (cholesterol)

## 6.0 INTERPRETATION OF RESULTS

### 6.1 EXPECTED VALUES

Guidelines for reference ranges have been suggested by the Panel of the National Institutes of Health's Cholesterol Consensus Development Conference and adopted by the National Cholesterol Education Program. In this unique effort, an institution's own reference range study may not be required. Consult your local accrediting agency, the college of American Pathologists, or the Joint Commission on Accreditation of Hospitals for guidance.

Total cholesterol (mg/dl)

Desirable	< 200
Borderline high	200-239
High	≥ 240

### 6.2 MEDICAL ALERT VALUES (10.7)

Each laboratory should establish low and high values beyond which the patient would require immediate attention by a physician. If a "medical alert value" is reached, always repeat the test to confirm the result and notify a physician if the result is confirmed.

### 6.3 LIMITATIONS OF PROCEDURE

Samples with bilirubin value in excess of 5 mg/dl will give low values by this method. Certain drugs may interfere with this assay or affect the circulating level of cholesterol (10.6).

## 7.0 QUALITY CONTROL

Standard practice for quality control should be applied to this system. Commercially available lyophilized controls can be used to monitor the daily acceptable variations. These controls should be traceable to NRS (National Reference System.) Normal and abnormal controls should be assayed at the beginning of each run, whenever a new reagent or a different lot number is being used, and following any system maintenance. The normal control should be in the range of 175-200 mg/dl. The abnormal control should be at the 240 mg/dl decision level. A satisfactory level of performance is achieved when the analyte values obtained are within the "acceptable range" established by the laboratory.

## 8.0 CALIBRATION PROCEDURES

The total cholesterol assay is calibrated by referencing the absorbance of the unknown sample to the absorbance of the calibrator. Refer to your instrument manual for more details. Calibration is required with the use of a new lot of reagent, any system maintenance or whenever indicated by quality control data.

## 9.0 PERFORMANCE CHARACTERISTICS

### 9.1 PRECISION

The estimates of precision shown below were obtained from assays of human control serum.

#### Within-Run

In this study, 15 replicates of 3 control sera were run.

	<u>Mean (mg/dl)</u>	<u>SD (mg/dl)</u>	<u>CV (%)</u>
Serum 1	242	± 2.18	0.90
Serum 2	214	± 0.97	0.45
Serum 3	144	± 1.54	1.07

#### Between-Run

In this study, 5 runs were made, each run consisting of 5 replicates of 4 control sera.

	<u>Mean (mg/dl)</u>	<u>SD (mg/dl)</u>	<u>CV (%)</u>
Serum 1	241	± 1.76	0.73
Serum 2	201	± 2.23	1.11
Serum 3	147	± 2.32	1.57
Serum 4	130	± 2.83	1.82

### 9.2 CORRELATION

A correlation study was done on the Technicon RA-500 system at 37° C comparing this method (y) and a similar comparative method (x<sub>1</sub>). The samples range between 97 mg/dl and 510 mg/dl. Another correlation study was done comparing this method (y) to a modified Abell-Kendall method (x<sub>2</sub>) run by a lipid reference laboratory. The samples range between 86 mg/dl and 319 mg/dl.

Number of Samples	Regression Equation <u>y=Biotron, x=Comparative</u>	Correlation Coefficient
30	y = .924 x <sub>1</sub> + 12.4	0.998
45	y = .990 x <sub>2</sub> + 3.46	0.981

Estimated Bias: The estimated bias for a selected decision level is computed based on the comparison with Abell-Kendall. Estimated Bias = (.990 - 1) decision level + 3.46. For decision levels 200 mg/dl and 240 mg/dl

<u>Decision level</u>	<u>Est. Bias</u>	<u>%</u>
200 mg/dl	1.46 mg/dl	0.73
240 mg/dl	1.06 mg/dl	0.44

### 9.3 RECOVERY

Cholesterol was added to 3 pools of fresh human serum to increase the cholesterol concentration by 50, 100 and 200 mg/dl. The recovery of the added cholesterol averaged 98.1%.

### 9.4 SENSITIVITY

A change in absorbance of 0.001A at 505nm at 37° C corresponds to approximately 0.56 mg/dl.

### 9.5 LINEARITY

This method is linear to 500 mg/dl. A sample with cholesterol beyond the linearity limit should be diluted 1 to 1 with 0.9% saline. Reassay the specimen and multiply the results by 2.

## 10.0 REFERENCES

- 10.1. Tonks, D.B., Clin. Biochem. 1, 12 (1967).
- 10.2. Zak, B., Clin. Chem. 23, 1201 (1977).
- 10.3. Allain, C.C., Poon, L., Chan, S.G., Richmond, W., Fu, P., Clin. Chem. 20, 470 (1974).
- 10.4. Roschlau, P., Bernt, E., Gruber, W., Z. Klin. Chem.. Klin. Blochem. 12, 226, (1974).
- 10.5. Witte, D.L., Barrett II, D.A., Wycoff, D.A., Clinical Chemistry 20, No. 10, 1282-1286 (1974).
- 10.6. Young, D.S., Effects of Drugs on Clinical Laboratory Tests, 3rd ed., Washington DC, AACC Press (1990).
- 10.7. G.J. Kost, "Critical Limits for Urgent Clinician Notification at U.S. Medical Centers"; JAMA, Feb. 2, 1990; Vol 263, No.5, p.704