CHOLESTEROL (CHOD-PAP)

Enzymatic Colorimetric Determination of Serum Cholesterol

REF. CHOL-MC – 0530 (5 x 30 ml)

INTENDED USE

NS Biotec cholesterol reagent is intended for the in vitro quantitative determination of total cholesterol in serum and plasma on both automated and manual systems.

CLINICAL SIGNIFICANCE

Cholesterol is a steroid with a secondary hydroxyl group in the C_3 position, and found in blood, bile, and brain tissue. It serves as a precursor to bile acids, steroids and vitamin D. It is synthesized in many types of tissue, but particularly in the liver and intestinal wall. Approximately, 75% of cholesterol is newly synthesized and a 25% originates from dietary intake. Measurements of serum cholesterol levels are important in the diagnosis and classification of hyperlipoproteinemias. Elevated cholesterol levels may occur with hypothyroidism, nephrotic syndrome, diabetes, and various liver diseases. There is a correlation between elevated serum cholesterol levels and the incidence of coronary artery diseases. Normal cholesterol levels are affected by stress, diet, age, gender, hormonal balance, and pregnancy. Depressed levels are associated with hyperthyroidism and severe liver diseases.

ASSAY PRINCIPLE

Cholesterol analysis was first reported by Liebermann in 1885 followed by Burchard in 1889. In Liebermann-Burchard reaction, cholesterol forms a blue-gree dye from polymeric unsaturated carbohydrates in an acetic acid/acetic anhydride/concentrated sulfuric acid medium. The Abell and Kendall method is specific for cholesterol but is technically complex and requires corrosive reagents. In 1974, Allain et al and Roeschlau et al were able to combine cholesterol esterase and cholesterol oxidase into a single enzymatic reagent for the determination of total cholesterol. NS Biotec cholesterol reagent combines the use of these enzymes with the peroxidase/phenol/4-aminoantipyrine system of Trinder[11] for the measurement of total cholesterol in human serum.
The series of reactions involved in the assay system are as follows:

1. Cholesterol esters are enzymatically hydrolyzed by cholesterol esterase (CE) to cholesterol and free fatty acids.
2. Free cholesterol, including that originally present, is then oxidized by cholesterol oxidase (CHOD) to cholest-4-en-3-one and H_2O_2.
3. In presence of peroxidase (POD), the formed hydrogen peroxide formed effects the oxidative coupling of phenol and 4-aminoantipyrine (4-AAP) to form a red-colored quinoneimine dye.

Cholesterol esters+H_2O $\xrightarrow{CHOD}$ **Cholesterol + fatty acids**

Cholesterol +O_2 $\xrightarrow{CE}$ **Cholest-4-ene-3-one+H_2O_2**

2 H_2O_2+ 4-AAP + Phenol $\xrightarrow{POD}$ **Quinoneimine dye + 4 H_2O**

The intensity of the color produced is directly proportional to cholesterol concentration. It is determined by measuring the increase in absorbance at 500 – 550 nm.

EXPECTED VALUES

Risk classification	Total Cholesterol
Desirable	<200 mg/dl (5.2 mmol/l)
Borderline high	200 – 239 mg/dl (5.2 – 6.2 mmol/l)
High	>240 mg/dl (6.2 mmol/l)

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range. For diagnostic purposes, the cholesterol results should always be assessed in conjunction with the patient's medical history, clinical examination, and other findings.

REAGENTS

Reagent Preparation & Stability

R_1	Cholesterol standard	200 mg/dl
R_2	Pipes buffer, pH 6.9	90 mmol/l
	Phenol	26 mmol/l
	Cholesterol oxidase	500 U/l
	Cholesterol esterase	500 U/l
	Peroxidase	1250 U/l
	4-Aminoantipyrine	0.4 mmol/l

All reagents are ready for use and stable up to the expiry date given on label when stored at 2–8^{0}C.

SPECIMEN

- Serum, or plasma.
- The only acceptable anticoagulants are heparin and EDTA.

Specimen Preparation & Stability

No special preparation of the patient is necessary, however it is recommended that prior to collection, patients should be following their usual diet and be in their usual state of health. Patients who are acutely ill, losing weight, pregnant or have had a myocardial infarction in the previous 3 months should be rescheduled.

Blood should be collected by venipuncture, after the patient has been in a seated position for at least 5 minutes. Tourniquet usage should be kept to a minimum and the specimen should be allowed to clot for 30 minutes at room temperature.

The best specimen is unhemolysed serum, and should be analyzed on the day of collection. When stored at 4^{0}C, specimens are stable for 3-4 days; specimens are stable at –20^{0}C for several months.

PROCEDURE

- **Manual Procedure**

Wavelength	500 - 550 nm
Cuvette	1 cm light path
Temperature	20-25 or 37 ^{0}C
Zero adjustment	against reagent blank
Specimen	Serum or plasma

	Blank	Standard	Specimen
R_2	1.0 ml	1.0 ml	1.0 ml
Standard	……	10 µl	……
Specimen	……	……	10 µl

Mix, incubate for 5 minutes at 37^{0}C or 10 minutes at 20-25^{0}C. Measure the absorbance of specimen ($A_{specimen}$) and standard ($A_{standard}$) against reagent blank.
The color is stable for 60 minutes.

- **Automated Procedure**

User defined parameters for different auto analyzers are available upon request

CALCULATION

Calculate the cholesterol concentration by using the following formulae:

Cholesterol Concentration=

$$\frac{\text{Absorbance of Specimen}}{\text{Absorbance of Standard}} \times \text{Standard value}$$

Unit conversion

mg/dl x 0.0259 = mmol/l

LINEARITY

When run as recommended, the assay is linear up to 800 mg/dl (20.7 mmol/l).

If result exceeds 800 mg/dl (20.7 mmol/l), specimen should be diluted with 0.9% NaCl solution and reassayed. Multiply the result by the dilution factor.

SENSITIVITY

The sensitivity is defined as the change of analytical response per unit change in analyte concentration at a pathlength of 1 cm.

When run as recommended the sensitivity of this assay is 3.0 mg/dl (0.08 mmol/l).

QUALITY CONTROL

It is recommended that controls (normal and abnormal) be included in:

- Each set of assays, or
- At least once a shift, or
- When a new bottle of reagent is used, or
- After preventive maintenance is performed or a clinical component is replaced.

Commercially available control material with established cholesterol values may be routinely used for quality control.

Failure to obtain the proper range of values in the assay of control material may indicate:

- Reagent deterioration,
- Instrument malfunction, or
- Procedure errors.

The following corrective actions are recommended in such situations:

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results on fresh control material still remain outside the limits, then repeat the test with fresh reagent.
- If results are still out of control, contact NS Biotec Technical Services.

INTERFERING SUBSTANCES

- **Anticoagulants**
  The only acceptable anticoagulants are heparin and EDTA.
- **Bilirubin:**
  Bilirubin levels higher than 7.5 mg/dl decrease the apparent total cholesterol concentration significantly.
- **Drugs:**
  Methyldopa causes artificially low total cholesterol values at the tested drug level. For a more comprehensive review of drugs affecting cholesterol assays refer to the publication by Young[13].
- **Haemoglobin:**
  No interference from haemoglobin up to a level of 500 mg/dl.
- **Lipemia:**
  No significant interference.
- **Others:**
  Ascorbic acid levels higher than 7.5 mg/dl decrease the apparent total cholesterol concentration significantly.
  Other 3-beta-hydroxysteroids cause positive interference but are not normally present in significant quantities in human serum[14].

WARNING & PRECAUTIONS

- NS Biotec cholesterol reagent is for in vitro diagnostic use only. Normal precautions exercised in handling laboratory reagents should be followed.
- Warm up working solution to the corresponding temperature before use.
- The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
- Valid results depend on an accurately calibrated instrument, timing, and temperature control.
- The reagent blank will not exceed an absorbance of 0.06 but don't use the reagent if it is turbid or if the absorbance is greater than 0.2 at 500 nm.

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	Consult Instruction for Use
	Caution Consult Accompanying Documents
IVD	In Vitro Diagnostic Medical Device
	Temperature Limitation
	Manufacturer
EC REP	Authorized Representative In The European Community
REF	Catalogue Number
LOT	Batch Code
	Use By

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