Cholesterol Reagent

PRODUCT SUMMARY

Stability	:	3 Months at 2-8°C
Linear Range	:	Up to 20 mmol/L (0 - 774 mg/dL)
Specimen Type	:	Serum
Method	:	Endpoint
Reagent Preparation	:	Add specified volume of distilled or deionised water.

SYMBOLS IN PRODUCT LABELLING

EC REP	Authorised Representative		Temperature Limitation
IVD	For in vitro diagnostic use		Use by/Expiration Date
LOT	Batch code/Lot number		CAUTION. CONSULT INSTRUCTIONS FOR USE.
REF	Catalogue number		Manufactured by
	Consult instructions for use		Xn - Harmful

INTENDED USE
This reagent is intended for the in vitro quantitative, diagnostic determination of cholesterol in human serum.

CLINICAL SIGNIFICANCE
Measurement of serum cholesterol levels can serve as an indicator of liver function, biliary function, intestinal absorption, propensity toward coronary artery disease, thyroid function and adrenal disease.
Cholesterol levels are important in the diagnosis and classification of hyperlipoproteinaemias. Stress, age, gender, hormonal balance and pregnancy affect normal cholesterol levels.[1,2]

METHODOLOGY
The use of enzymes to assay cholesterol has been studied by many investigators.[3,4] This reagent is based on the formulation of Allain et al[5] and the modification of Roeschlau[6] with further improvements to render the reagent stable in solution.

1. Cholesterol Esters $\xrightarrow{CE}$ Cholesterol + Fatty Acids

2. Cholesterol + O_2 $\xrightarrow{CO}$ Cholest-4-en-3-one + H_2O_2

3. $2H_2O_2$ + HBA +4 AAP $\xrightarrow{POD}$ Quinoneimine Dye + $4H_2O$

Where:

CE	=	Cholesterol Esterase
CO	=	Cholesterol Oxidase
HBA	=	Hydroxybenzoic Acid
4AAP	=	4-aminoantipyrine
POD	=	Peroxidase

1. Cholesterol esters are enzymatically hydrolysed by cholesterol esterase to cholesterol and free fatty acids.
2. Free cholesterol, including that originally present, is then oxidized by cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide.
3. The hydrogen peroxide combines with HBA and 4-aminoantipyrine to form a chromophore (quinoneimine dye) which may be quantitated at 500-550 nm. For bichromatic analysers the blank wavelength should be set to 600 or 660 nm.

REAGENT COMPOSITION

Active Ingredients	Concentration
Cholesterol oxidase (microbial)	> 100 U/L
Cholesterol Esterase (microbial)	> 250 U/L
Peroxidase (Horseradish)	> 150 U/L
4-aminoantipyrine	0.25 mmol/L
HBA	10 mmol/L
Buffer	50 mmol/L
Surfactants	
pH 6.7 ± 0.1 at 20°C	

WARNING: Do not ingest. Avoid contact with skin and eyes. If spilt thoroughly wash affected area with water. Reagent contains sodium azide which may react with copper or lead plumbing. Flush with plenty of water when disposing. For further information consult the Cholesterol reagent Material Safety Data Sheet. The Packaging of This Product Contains Dry Natural Rubber. Exercise precaution when handling crimps and broken glass vials, as sharp edges can injure the user.

R22	Harmful if swallowed.
R36/37/38	Irritating to eyes, respiratory system and skin.
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

REAGENT PREPARATION
Reconstitute the reagent with the volume of distilled or deionised water stated on the vial label.

STABILITY AND STORAGE
Prior to use:
When stored refrigerated at 2-8°C the reagent is stable until the expiry date stated on the bottle and kit box label.
Reconstituted Reagent:
When stored capped at 2-8°C, the reagent is stable for at least 3 months.
Indications of Reagent Deterioration:
- Turbidity;
- Reagent Absorbance > 0.2 AU at 500 nm; and/or
- Failure to recover control values within the assigned range.

SPECIMEN COLLECTION AND HANDLING
Collection:[8] 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.
Serum: The best specimen is non-haemolysed serum collected as per the above instructions.
Storage:[2] Specimens should be analysed on the day of collection. When stored at 4°C, specimens are stable for 3-4 days. Specimens are stable at -20°C for several months.

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED
- If required, pipettes for accurately dispensing measured volumes.
- A clinical chemistry analyser capable of maintaining constant temperature (37°C) and measuring absorbance between 500 and 550 nm.
- Analyser specific consumables, eg: sample cups.
- Normal and Abnormal assayed controls.
- Calibrator traceable to NRS/CHOL material.

ASSAY PROCEDURE
The following system parameters are recommended. Individual instrument applications are available upon request from the Technical Support Group.

SYSTEM PARAMETERS

Temperature	30/37°C
Primary Wavelength	500 nm (500 - 550nm)
Secondary Wavelength	660 nm (600 - 660nm)
Assay Type	End Point
Direction	Increase
Sample:Reagent ratio	1:100
e.g.Sample vol	3 µL
Reagent vol	300 µL
Incubation Time	300 seconds
Reagent Blank Limits	Low 0.0 AU
(500nm, 1cm lightpath)	High 0.2 AU
Linearity	0 - 20 mmol/L (0 - 774 mg/dL)
Sensitivity	62 ΔmA per mmol/L
(500nm, 1cm lightpath)	(1.6 ΔmA per mg/dL)

CALCULATIONS
Results are calculated, usually automatically by the instrument, as follows:

$$\text{Cholesterol} = \frac{\Delta\text{Abs/min of Unknown}}{\Delta\text{Abs/min of Calibrator}} \times \text{Calibrator Value}$$

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Example:

Absorbance of calibrator = 0.28
Absorbance of unknown = 0.19
Value of calibrator = 5.5 mmol/L (213 mg/dL)

$$\text{Cholesterol} = \frac{0.19}{0.28} \times 5.5 = 3.73 \text{ mmol/L}$$

$$\text{Cholesterol} = \frac{0.19}{0.28} \times 213 = 145 \text{ mg/dL}$$

NOTES

1. Specimens with cholesterol values greater than 20 mmol/L (774 mg/dL) should be diluted and reassayed. Multiply the results by the dilution factor.
2. The assay can be performed at 30°C by increasing the incubation time to 10 minutes or at 25°C by incubating for 15 minutes.
3. The colour development is stable for 30 minutes.
4. Unit conversion mmol/L x 38.7 = mg/dL.

CALIBRATION

Calibration is required. A suitable aqueous standard or serum based calibrator traceable to NRS/CHOL material is recommended. Appropriate calibrator levels range from 5.2 to 7.8 mmol/L (200 - 300 mg/dL).
For Calibration Frequency on automated instruments refer to the instrument manufacturers specifications. However, calibration stability is contingent upon optimum instrument performance and the use of reagents which have been stored as recommended in the stability and storage section of this package insert.
Recalibration is recommended at anytime if one of the following events occurs:

- The lot number of reagent changes.
- Preventative maintenance is performed or a critical component is replaced.
- Control values have shifted or are out of range and a new vial of control does not rectify the problem.

QUALITY CONTROL

To ensure adequate quality control, two levels of control, one in the normal range (4.5 - 5.2 mmol/L or 175 - 200 mg/dL), and one at the high level (6.2 - 6.7 mmol/L or 240 - 260 mg/dL) should be run as unknown samples:-

- At least every eight hours.
- When a new bottle of reagent is used.
- After preventative maintenance is performed or a critical component is replaced.

Control results falling outside the upper or lower limits of the established ranges indicate the assay may be out of control. 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 are still out of control, recalibrate with fresh calibrator, then repeat the test.
- If results are still out of control, perform a calibration with freshly prepared reagent, then repeat the test.
- If results are still out of control, contact Technical Services or the local distributor.

LIMITATIONS

1. Studies to determine the level of interference from haemoglobin, bilirubin and lipaemia were carried out. The following results were obtained:
   Haemoglobin: No interference from haemoglobin up to 500 mg/dL.
   Free Bilirubin: No interference from bilirubin up to 182µmol/L (10.6 mg/dL).
   Conjugated Bilirubin: No interference from bilirubin up to 58µmol/L (3.4 mg/dL).
   Lipaemia: No interference from lipaemia, measured as absorbance at 630nm up to 1.68 AU.
2. Ascorbic acid at high abnormal levels may cause negative interference.
3. Other 3-beta-hydroxysteroids cause positive interference but are not normally present in significant quantities in human serum.
4. For a more comprehensive review of factors affecting cholesterol assays refer to the publication by Young.[7]

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EXPECTED VALUES

The following values are those recommended by the US National Cholesterol Education Program Expert Panel.[8]

Desirable blood Cholesterol	< 5.2 mmol/L (200mg/dL)
Borderline high blood Cholesterol	5.2 - 6.1 mmol/L (200 - 239 mg/dL)
High Blood Cholesterol	≥ 6.2 mmol/L (240 mg/dL)

PERFORMANCE DATA

The following data was obtained using the Cholesterol reagent on a well maintained automated clinical chemistry analyser. Users should establish product performance on their specific analyser used.

IMPRECISION

Imprecision was evaluated using two levels of commercial control.

		Level I	Level II
	Number of samples	20	20
Within Run:	Mean (mmol/L / mg/dL)	3.08 / 119	7.15 / 277
	SD (mmol/L / mg/dL)	0.09 / 3.4	0.05 / 1.9
	C.V. (%)	2.89	0.70
		Level I	Level II
	Number of samples	40	40
Between Run:	Mean (mmol/L / mg/dL)	3.13 / 121	6.94 / 269
	SD (mmol/L)	0.13 / 4.9	0.27 / 10.5
	C.V. (%)	4.04	3.89

ACCURACY

Comparison studies were carried out using a similar commercially available Cholesterol reagent as a reference. Calibrations were carried out using material with a cholesterol value traceable to the WHO lipid standardisation laboratory at Centres for Disease Control. Serum samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained.

Number of sample pairs	48
Range of sample results	2.4 - 14.7 mmol/L (93 - 569 mg/dL)
Mean of reference method results	5.7 mmol/L (221 mg/dL)
Mean of Cholesterol results	5.9 mmol/L (228 mg/dL)
Slope	0.985
Intercept	0.24 mmol/L (9.3 mg/dL)
Correlation coefficient	0.985

LINEARITY

When run as recommended the assay is linear between 0 and 20 mmol/L (0 - 774 mg/dL).

SENSITIVITY

When run as recommended the sensitivity of this assay is 62 ΔmAbs per mmol/L or 1.6 ΔmAbs per mg/dL (1cm light path, 500nm).

REFERENCES

1. Searcy R L. "Diagnostic Biochemistry" McGraw-Hill, New York, NY. 1969.
2. Tietz Textbook of Clinical Chemistry. Burtis CA and Ashwood ER (Eds). Second Edition, WB Saunders Company, 1994.
3. Flegg H M. Ann. Clin. Biochem. 1973; 10:79.
4. Richmond W. Clin. Chem. 1973; 19:1350-1356.
5. Allain C C, Poon L S, Chan C S G, Richmond W and Fu P C. Clin Chem. 1974; 20: 470-475.
6. Roeschlau P, Bernt E and Gruber W A Clin. Chem Clin Biochem 1974; 12: 226.
7. Young D S et al. Clin. Chem 1975; 21.
8. NCEP Expert Panel. Arch Intern Med 1988; 148: 36-69.

REF

Reorder Information

Catalogue No.	Configuration
TR13315	20 x 20 mL
TR13303	10 x 50 mL