

Infinity™

Cholesterol Liquid Stable Reagent

PRODUCT SUMMARY

Stability	:	Until Expiry at 2-8°C
Linear Range	:	Up to 20 mmol/L (774 mg/dL)
Specimen Type	:	Serum or Plasma
Method	:	Endpoint
Reagent Preparation	:	Supplied ready to use.

IVD

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		

INTENDED USE

This reagent is intended for the in vitro quantitative determination of cholesterol in human serum or plasma.

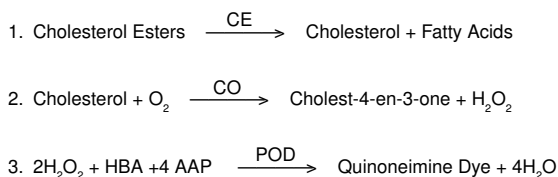
CLINICAL SIGNIFICANCE

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 al⁵ and the modification of Roeschlau⁶ with further improvements to render the reagent stable in solution.



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

	<u>Concentration</u>
Cholesterol oxidase (microbial)	> 200 U/L
Cholesterol Esterase (microbial)	> 500 U/L
Peroxidase (Horseradish)	> 300 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 Infinity Cholesterol Liquid Stable Reagent Material Safety Data Sheet.

REAGENT PREPARATION

Reagent is supplied ready to use.

STABILITY AND STORAGE

When stored refrigerated at 2-8°C in sealed air-tight containers and protected from light the reagent is stable until the expiry date on the bottle.

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:⁷ 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.

Plasma: Hepranised plasma is a suitable specimen.

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

- 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 control Material.
- 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)
Analytical 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:

$$\text{Cholesterol} = \frac{\text{Absorbance of Unknown}}{\text{Absorbance of Calibrator}} \times \text{Calibrator Value}$$

Example:

Absorbance of calibrator	=	0.42
Absorbance of unknown	=	0.25
Value of calibrator	=	7.0 mmol/L (271 mg/dL)

$$\text{Cholesterol} = \frac{0.25}{0.42} \times 7.0 = 4.2 \text{ mmol/L}$$

$$\text{Cholesterol} = \frac{0.25}{0.42} \times 271 = 161 \text{ mg/dL}$$

NOTES

- Specimens with cholesterol values greater than 20 mmol/L (774 mg/dL) should be diluted and reassayed. Multiply the results by the dilution factor.
- 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.
- The colour development is stable for 30 minutes.
- 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 - 250 mg/dL) should be run as unknown samples:-

- At least every eight hours or as established by the laboratory.
- When a new bottle of reagent is used.
- After preventative maintenance is performed or a critical component is replaced.
- With every calibration.

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 fresh reagent, then repeat the test.
- If results are still out of control, contact Technical Services or the local distributor.

LIMITATIONS

- 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 182 µmol/L (10.6 mg/dL).
Conjugated Bilirubin: No interference from bilirubin up to 58 µmol/L (3.4 mg/dL).
Lipaemia: No interference from lipaemia, measured as absorbance at 630 nm up to 1.68 AU.
- Ascorbic acid at high abnormal levels may cause negative interference.⁸
- Other 3-beta-hydroxysteroids cause positive interference but are not normally present in significant quantities in human serum.⁸
- For a more comprehensive review of factors affecting cholesterol assays refer to the publication by Young.⁹

EXPECTED VALUES

The following values are those recommended by the US National Cholesterol Education Program Expert Panel.⁷

Desirable blood Cholesterol	< 5.2 mmol/L (200 mg/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 Infinity Cholesterol Liquid Stable 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 and following the NCCLS EP5-T procedure¹⁰.

	Level I	Level II
Number of samples	20	20
Mean (mmol/L / mg/dL)	3.79 / 147	6.76 / 262
Within run: SD (mmol/L / mg/dL)	0.08 / 3.1	0.11 / 4.1
C.V. (%)	2.8	1.5
Total: SD (mmol/L / mg/dL)	0.11 / 4.1	0.10 / 3.9
C.V. (%)	2.8	1.5

METHOD COMPARISON

Comparison studies were carried out using a similar commercially available Cholesterol reagent as a reference. Calibrations were carried out using material 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	60
Range of sample results	0.1-12.0 mmol/L (4-464 mg/dL)
Mean of reference method results	5.7 mmol/L (221 mg/dL)
Mean of Cholesterol Infinity results	5.7 mmol/L (221 mg/dL)
Slope	0.989
Intercept	-0.02 mmol/L (-0.8 mg/dL)
Correlation coefficient	0.999

LINEARITY

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

ANALYTICAL 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

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REF


Reorder Information

Catalogue No.

2350-400H
 TR13421/2350-250
 2350-500
 7500-012A
 TL13401
 TY13401

Configuration

4 x 100 mL (Hitachi)
 2 x 125 mL
 2 x 250 mL
 4 x 500 mL
 8 x 100 mL (ILab 600)
 4 x 50 mL (Hitachi)

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