Pointe Cholesterol Reagent Set

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Intended Use
For the quantitative determination of Total Cholesterol in serum using the Yumizen C230 and Yumizen C240 analyzers. **Rx Only.**

Method History
A Cholesterol method developed in the late 1800's by Lieberman[1] and Burchard[2] is still in use today despite its corrosive nature and its susceptibility to many interfering substances.
Work on an enzymatic procedure was begun by Flegg[3] and Richmond[4] in the early 70's. Allain[5] and Roeschlau[6] began using cholesterol esterase and oxidase, in a single reagent to determine total cholesterol in serum.
Trinder's[7] color system of peroxidase/phenol/4-aminoantipyrine has been used successfully for some time now. With appropriate calibrator value assignment, this method has been shown to provide excellent accuracy in relation to the reference methodology.

Principle
C. Esterase
Cholesterol Esters ----------➢ Cholesterol + Fatty Acids

C. Oxidase
Cholesterol + O_2 ----------➢ Cholesterol-3-one + H_2O_2

Peroxidase
$2H_2O_2$ + 4-AAP + Phenol ----------➢ Quinoneimine + 4 H_2O
(red dye)

The intensity of the red color produced is directly proportional to the total cholesterol in the sample when read at 500nm.

Reagents
4-Aminoantipyrine 0.25mM, Cholesterol Esterase >150u/L, Cholesterol Oxidase >150u/L, Peroxidase >1500u/L, Phenol >15mM, Phosphate Buffer, pH 6.8, non-reactive stabilizers and preservatives.

Reagent Preparation
The reagent is ready to use.

Reagent Storage
1. Store reagent at 2-8°C.
2. The reagent is stable until the expiration date when stored at 2-8°C.

Reagent Deterioration
Do not use if:
1. The reagent is turbid.
2. The reagent does not meet stated performance parameters.

Precautions
1. This reagent is for *in vitro* diagnostic use only.
2. Not to be used internally in humans or animals. Normal precautions for handling laboratory reagents should be followed.
3. Additional safety information concerning storage and handling of this product is in the Material Safety Data Sheet for this product.

Specimen Collection and Storage
Nonhemolyzed serum is recommended. Cholesterol in serum is reported stable for seven days at room temperature (18-25°C) and six months when frozen and properly protected against evaporation.[8,9]

Interferences
A number of drugs and substances affect concentrations of cholesterol. See Young, et al.[10]

Materials Provided
Cholesterol Reagent

Materials Required but not Provided
1. Yumizen C230 / Yumizen C240 Analyzer
2. Yumizen C230 / Yumizen C240 Operation manual
3. Chemistry Calibrator, catalog number C7506-50
4. Chemistry Control, catalog number C7592-100

Test Parameters

Test:	Chol	Chemistry:	Cholesterol
Chemistry No.:	210	Print Name:	Chol
Reaction Type:	Endpoint	Reaction Direction:	Positive
Pri. Wave:	510 nm	Sec. Wave:	670 nm
Decimal.:	0	Samp. Type:	Serum
Blank Time:		Reaction Time:	18 20
Unit:	mg/dL	Incubation Time:	0

	Sample Vol.	Aspirated	Diluent		Reagent Vol.	Diluent	
Standard;	2	uL	uL	ulL	R1: 200	uL	uL
Decreased;		uL	uL	ulL			
Increased;		uL	uL	ulL			

Linearity Range (Standard); 0-500			Linearity Limit:	
Linearity Range (Decreased):			Substrate Depletion:	
Linearity Range (Increased):			Mixed Blank Abs.:	- 40000 40000
R1 Blank Abs.:	- 40000	40000	On-board Stability:	30 Day (s)
Blank Response	- 40000	40000	Reagent Alarm Limit:	5
Twin Chemistry:				

Prozone Check:
Q1: Q2: Q3:
Q4: PC: ABS:

Use Qualitative Result:
Range: Flag:

Slope Offset:

Slope	Offset	Unit
1	0	mg/dL

Pretreatment:
Pretreat Sample Vol.: uL Pretreat Reagent Vol.: uL

Ref. Range:
Sample Type: Gender: Age Range: Ref. Range: Critical Range: Unit:

Calibration Setup Parameters

Chem: Chol
Calibration Setting
Math Model: Two-Point Linear
Factor: Replicates: 2
Acceptance Limits
Cal Time: 336 hr.
Slope Diff: SD:
Sensitivity: Repeatability:
Deter Coeff:
Auto Calib.
☐ Cal Time

Calibrator	Conc.	Pos	Lot No.
Water	0.0	W	
Chem Cal	*	*	

* User Defined

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Limitations
Samples with values exceeding 500 mg/dl should be diluted 1:1 with saline and re-run. The final answer should be multiplied by two.

Calibration
Use an NIST-traceable serum calibrator. The procedure should be calibrated according to the instrument manufacturer's instructions. If control results are found to be out of range, the procedure should be re-calibrated.

Calculation (Example)
Abs. = Absorbance

$$\frac{\text{Abs. (Patient)}}{\text{Abs. (Standard)}} \times \text{Concentration of Std.} = \text{Cholesterol (mg/dl)}$$

Example: Abs. (Patient) = 0.40, Abs. (Standard) = 0.32, Concentration of Standard = 200 mg/dl

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

Quality Control
Serum controls with known normal and elevated values should be run routinely to monitor the validity of the reaction. These controls should be run at least with every working shift in which Cholesterol assays are performed. It is recommended that each laboratory establish its own frequency of control determination. Quality control requirements should be performed in conformance with local, state, and/or Federal regulations or accreditation requirements.

Expected Values[11]
Recommended Range:

Desirable Cholesterol:	<200mg/dl
Borderline-High Cholesterol:	200-239mg/dl
High Cholesterol:	>240mg/dl

Performance
1. Linearity: 500 mg/dl
2. Comparison: A study was performed between the Yumizen 200 series and a similar analyzer using this method, resulting in a correlation coefficient of y = 1.068x - 2.5 with a correlation coefficient of 0.968.
3. Precision: Precision studies were performed using the Yumizen 200 series analyzer following a modification of the guidelines which are contained in NCCLS document EP5-T2.[12]

Within Run			Day to Day		
Mean	**S.D.**	**C.V.%**	**Mean**	**S.D.**	**C.V.%**
140.4	3.1	2.2	123.1	3.0	2.4
269.1	4.0	1.5	251.4	6.9	2.7

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

References

1. Lieberman, C., Ber. 18:1803 (1885).
2. Burchard, H., Chem. Fentr. 61:25 (1890).
3. Flegg, H.M., Ann. Clin. Biochem. 10:79 (1973).
4. Richmond, W., Scand. J. Clin. Lab. Invest. 29:Suppl. 26, abstr. 3:25 (1972).
5. Allain, C.C., et al, Clin. Chem. 20:470 (1974).
6. Roeschlau, P., et al, Z. Klin. Chem. Klin. Biochem 12:226 (1974).
7. Trinder, P., Ann. Clin. Biochem. 6:24 (1969).
8. Perlstein, M.T., et al, J. Microchem. 22:403 (1977).
9. Witte, D.L., et al, Clin. Chem. 20:1282 (1974).
10. Young, D.S. et al, Clin. Chem. 21:1D (1975).
11. National Institute of Health Publication No. 88-2926 "Detection, Evaluation, and Treatment of High Cholesterol in Adults", November (1987).
12. NCCLS document "Evaluation of Precision Performance of Clinical Chemistry Devices", 2nd Ed. (1992).

Symbol Key
Use by (YYYY-MM-DD)	LOT Lot and batch code
REF Catalog number	Manufacturer
IVD *In vitro* diagnostic medical device	Temperature limitation
Consult instructions for use	**Rx Only:** Prescription Use Only
CE mark	EC REP Authorized representative in the European Community

REF 12-C7510-160 Manufactured by HORIBA Instruments Incorporated - Pointe Brand 5449 Research Drive Canton, MI 48188 IVD

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Certified to Perform Reagents
The Pointe reagents are certified to be manufactured according to specified parameters. Any Pointe reagent product not meeting specifications through its listed expiration date will be remedied immediately without charge.

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