CHOLESTEROL (CHOL)

Enzymatic Endpoint Method
RX DAYTONA PLUS

INTENDED USE

For the quantitative *in vitro* determination of cholesterol in serum and plasma.

Cholesterol measurements are used in the diagnosis and treatments of disorders involving excess cholesterol in the blood and lipid and lipoprotein metabolism disorders.

FOR PRESCRIPTION USE ONLY.

Cat. No.		
CH 8310	R1. Reagent	4 x 20 ml

GTIN: 05055273208474

CLINICAL SIGNIFICANCE[1,2,3]

Cholesterol measurements are used in the diagnosis and treatments of lipid lipoprotein metabolism disorders. Lipids play an important role in the body; they serve as hormones or hormone precursors, aid in digestion, provide energy, storage and metabolic fuels, act as functional and structural components in biomembranes and form insulation to allow nerve conduction and prevent heat loss.

In clinical chemistry, over the last decade however, lipids have become associated with lipoprotein metabolism and atherosclerosis. The Abell Kendell method, reported by Abell *et al* (1952) involved extraction of cholesterol by organic solvents and subsequent alkaline hydrolysis of the cholesterol esters. This reaction is highly specific but the reagents involved are corrosive and the method cumbersome, rendering it impractical for routine laboratory use.

The use of cholesterol oxidase following specimen saponification as described by Richmond (1973) provided the first step toward a totally enzymatic procedure. In 1974 Allain *et al*. and Roeschlaw *et al*. published the first fully enzymatic procedure for cholesterol determinations replacing chemical saponification with enzymatic saponification.

ASSAY PRINCIPLE[4]

The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase.

$$\text{Cholesterol ester} + H_20 \xrightarrow[\text{esterase}]{\text{Cholesterol-}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + 0_2 \xrightarrow[\text{oxidase}]{\text{Cholesterol}} \text{Cholestene-3-one} + H_20_2$$

$$2H_20_2 + \text{phenol} + \text{4-Aminoantipyrine} \xrightarrow{\text{peroxidase}} \text{quinoneimine} + 4H_20$$

SPECIMEN COLLECTION AND PREPARATION[5]

Serum: may be used.
Plasma: K_2 EDTA or heparin may be used. Do not use citrate, oxalate or fluoride.
Plasma and Serum samples may be stored for up to 4 days at +4°C.

REAGENT COMPOSITION

Contents		Concentrations in the Test
R1.	**Reagent**	
	4-Aminoantipyrine	0.25 mmol/l
	Phenol	6.00 mmol/l
	Peroxidase (E.C.1.11.1.7, Horse Radish, +25°C)	≥ 0.50 U/ml
	Cholesterol esterase (E.C.3.1.1.13. *Pseudomonas*, +37°C)	≥ 0.20 U/ml
	Cholesterol oxidase (E.C.1.1.3.6. *Nocardia*, +37°C)	≥ 0.10 U/ml
	Sodium Azide	0.09%

SAFETY PRECAUTIONS AND WARNINGS

For *in vitro* diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.

Solution R1 contains Sodium Azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.

Sodium Azide reacts with lead and copper plumbing, to form potentially explosive azides. When disposing of such reagents, flush with large volumes of water to prevent azide build up. Exposed metal surfaces should be cleaned with 10% sodium hydroxide.

Please dispose of all Biological and Chemical materials according to local guidelines.

Safety Data Sheets are available on request.

The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.

STABILITY AND PREPARATION OF REAGENT

R1. Reagent

Contents ready for use. The reagent is stable up to the expiry date when stored at +2°C to +8°C in the absence of contamination, protected from light.

MATERIALS PROVIDED

Cholesterol Reagent

MATERIALS REQUIRED BUT NOT PROVIDED

Randox Assayed Multisera Level 2 (Cat. No. HN 1530) and Level 3 (Cat. No. HE 1532)
Randox Calibration Serum Level 3 (Cat. No. CAL 2351)
RX **series** Saline (Cat. No. SA 8396)

RX DAYTONA PLUS CALIBRATION

0.9% NaCl as zero calibrator and Randox Calibration Serum Level 3 are recommended for calibration. A 2-point calibration is recommended.

PROCEDURE NOTES

This Cholesterol assay is susceptible to carryover between the following assays, when run on the same system: Calcium, Magnesium and Lipase.
To avoid the potential for carryover, please refer to the RX Instrument Carryover Avoidance document - prior to sample analysis - to confirm the test running order and for details of recommended washes.

To access the RX Instrument Carryover Avoidance document, please visit www.randox.com. Select **Support & Documentation**, followed by **Reagent Product Inserts**.
Please note: You will be required to create a user account if you do not already have one. To do this, please select **Request Access** and complete all the fields displayed.
Once logged in, please enter "Carryover" in the **Search** field, and the Carryover Avoidance document will be displayed.
Alternatively, please contact Randox Laboratories Technical Services, Northern Ireland +44 (0) 28 9445 1070 or technical.services@randox.com.

The Chemistry parameters for Randox Dedicated RX **daytona plus** Assays are predefined on the hard drive of the analyser PC. The required programs should be downloaded to the analyser software. Please note that the predefined chemistry parameters use SI units. If alternative units are required, these can be edited by the user. In this case, the technical range should be edited in accordance with the users selected units. All necessary instructions are encoded on the bar code. If the barcode cannot be read by the analyser, enter manually the series of numbers given beneath the barcode. If problems continue, contact Randox Laboratories Customer Technical services, Northern Ireland +44 (0) 28 9445 1070 or USA Customer Support 1-866-472-6369

STANDARDISATION

Randox Calibration Serum Level 3 is traceable to Cholesterol reference material NIST909.

This Cholesterol assay has not been CRMLN certified.

QUALITY CONTROL

Randox Assayed Multisera, Level 2 and Level 3 are recommended for daily quality control. Two levels of controls should be assayed at least once a day. Values obtained should fall within a specified range. If these values fall outside the range and repetition excludes error, the following steps should be taken:

1. Check instrument settings and light source.
2. Check cleanliness of all equipment in use.
3. Check water, contaminants i.e. bacterial growth may contribute to inaccurate results.
4. Check reaction temperature.
5. Check expiry date of kit and contents.
6. Contact Randox Laboratories Technical services, Northern Ireland +44 (0) 28 9445 1070 or USA Customer Support 1-866-472-6369

Quality control requirements should be determined in conformance with government regulations or accreditation requirements.

SPECIFICITY/LIMITATIONS

Cholesterol oxidase from *Norcardia* is not absolutely specific for cholesterol as it will oxidise several cholesterol analogues such as dihydrocholesterol or 7-dehydrocholesterol. Since these derivatives do not exist in serum in significant concentrations, the cholesterol oxidase from *Nocardia erythropolis* is suitable for a reliable cholesterol determination.

NORMAL VALUES IN SERUM/PLASMA[6]

Risk levels

Value	Interpretation
< 200 mg/dl (5.17 mmol/l)	Desirable blood cholesterol
200 - 239 mg/dl (5.17 - 6.18 mmol/l)	Borderline-high blood cholesterol
≥ 240 mg/dl (6.20 mmol/l)	High blood cholesterol

SPECIFIC PERFORMANCE CHARACTERISTICS

The following performance characteristics were obtained using a RX **daytona plus** analyser.

INTERFERENCE

The following analytes were tested up to the levels indicated at Cholesterol concentrations of 150 mg/dl and 250 mg/dl and found not to interfere:

Haemoglobin	No significant interference up to 750mg/dL
Total Bilirubin	No significant interference up to 60mg/dL
Conjugate Bilirubin	No significant interference up to 60mg/dL
Intralipid®	No significant interference up to 1000mg/dL
Ascorbic Acid	No significant interference up to 6mg/dL

Physiological changes in serum or plasma analyte concentrations can be caused by a number of substances. Comprehensive discussion of possible interfering substances, their serum or plasma concentrations, and their possible physiological involvements is beyond the scope of this document. The listed reference contains specific details on known potential interfering substances[7]. The user must remain vigilant to the possible effect on results of unknown interferences from medications or endogenous substances. All patient results must be evaluated in light of the total clinical status of the patient.

REPORTABLE RANGE

Linearity data demonstrates that the reportable range for Cholesterol on the RX **daytona plus** is 11.72 to 618 mg/dl (0.30 to 16.0 mmol/l).

SENSITIVITY

The limit of Quantitation (LoQ), the limit of Detection (LoD) and the limit of Blank (LoB) were determined consistent with CLSI guidelines EP17-A2. LoQ is the lowest concentration that can be detected with ≤20% imprecision. LoD is the lowest concentration that can be detected to determine the presence or absence of Cholesterol. LoB is the highest concentration that is likely to be observed in a blank sample.

RX daytona plus

Limit of Blank (mg/dl)	3.1
Limit of Detection (mg/dl)	6.31
Limit of Quantitation (mg/dl)	11.72

PRECISION

Precision estimates were completed according to CLSI documents EP5-A2. Each sample was assayed in duplicate twice per day for 20 days.

Within Run Precision

	Level 1	Level 2	Level 3	Level 4
Mean (mg/dl)	177	228	272	592
SD	3.56	4.04	3.84	6.76
CV(%)	2.0	1.8	1.4	1.1
n	80	80	80	80

Total Precision

	Level 1	Level 2	Level 3	Level 4
Mean (mg/dl)	177	228	272	592
SD	4.82	6.20	7.35	11.3
CV(%)	2.7	2.7	2.7	1.9
n	80	80	80	80

CORRELATION

This method (Y) was compared with another commercially available method (X) and the following linear regression equation obtained:

Y = 1.00X – 4.77
and a correlation coefficient of r = 0.997
107 patient samples were analysed spanning the range 25 to 599 mg/dl.

MATRIX COMPARISON

Lithium Heparin

Patient samples were drawn in matched pairs – one sample serum (x) and the second sample lithium heparin plasma (y). A minimum of 54 matched patient sample pairs were analysed in singlicate spanning the range 25 to 613 mg/dl and the following linear regression equation was obtained:

y = 1.01x – 6.54
Correlation coefficient of r = 0.997

Potassium EDTA

Patient samples were drawn in matched pairs – one sample serum (x) and the second sample potassium EDTA plasma (y). A minimum of 50 matched patient sample pairs were analysed in singlicate spanning the range 29 to 603 mg/dl and the following linear regression equation was obtained:

y = 0.99x + 2.85
Correlation coefficient of r = 0.998

REFERENCES

1. Abell, L.L, Levey, B.B., Brodie B.B., *et al*, *J. Biol Chem* **195** : 357, 1952.
2. Richmond, N., *Clin Chem* **19** : 1350 - 1356, 1973.
3. Roeschlau, P., Bernt, E. and Gruber, J.W., *Clin Chem Clin Biochem*. **12** : 403, 1974.
4. Trinder, P., Ann clin Biochem **6** : 24, 1969.
5. Clinical Laboratory Diagnostics. 1st Edition (1998) p169; Lothar Thomas ed. TH-Books Verlagsgesellschaft mbH, Frankfurt/Main, Germany.
6. Third Report of the National Cholesterol Education Programme (NCEP) Expert Panel on Detection, Evaluation and treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA Publication, Vol 285, No. 19, P2486 - 2497; 2001.
7. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Washington, DC: AACC Press; 2000.

The presence of a vertical bar in the margin indicates a technical update from the previous revision.

EC REP

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