BECKMAN COULTER

CE

AU

Instructions For Use

CHOL
CHOLESTEROL

REF OSR6116 4 x 22.5 mL R1
OSR6216 4 x 45 mL R1
OSR6616 4 x 173 mL R1

For *in vitro* diagnostic use only.

PRINCIPLE

INTENDED USE

Enzymatic colour test for the quantitative determination of cholesterol in human serum and plasma on Beckman Coulter AU analysers.

OSR6616 for use on the AU5800, AU2700 and AU5400 systems only.

SUMMARY AND EXPLANATION

Reference[1]

Cholesterol is synthesised ubiquitously throughout the body and is an essential component of cell membranes and lipoproteins as well as being a precursor for the synthesis of steroid hormones and bile acids.

The individual predictive value of total cholesterol concentration with regard to coronary risk is low. Cholesterol is mainly transported in two lipoprotein classes (LDL and HDL), both of which play a contradictory role in the pathogenesis of lipid disorders. The total cholesterol concentration therefore provides only a baseline value that indicates whether further laboratory investigations of lipoprotein metabolism should be carried out (HDL, LDL, and triglycerides).

METHODOLOGY

Reference[2]

The Cholesterol reagent utilises an enzymatic method to measure cholesterol in human serum and plasma. In this procedure cholesterol esters in a sample are hydrolysed by cholesterol esterase (CHE). The free cholesterol produced is oxidised by cholesterol oxidase (CHO) to cholestene-3-one with the simultaneous production of hydrogen peroxide (H_2O_2), which oxidatively couples with 4-aminoantipyrine and phenol in the presence of peroxidase (POD) to yield a chromophore.

The red quinoneimine dye formed can be measured spectrophotometrically at 540/600 nm as an increase in absorbance.

CHEMICAL REACTION SCHEME

$$\text{2 Cholesterol esters} + 2\ H_2O \xrightarrow{\text{CHE}} \text{2 Cholesterol} + \text{2 Fatty acids}$$

$$\text{2 Cholesterol} + 2\ O_2 \xrightarrow{\text{CHO}} \text{2 Cholestene-3-one} + 2\ H_2O_2$$

$$2\ H_2O_2 + \text{4-Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinoneimine} + 4\ H_2O$$

SPECIMEN

TYPE OF SPECIMEN

Reference[3]

Serum and EDTA or heparinised plasma. Icteric samples should be avoided.

Plasma is not recommended using anticoagulants such as oxalate, citrate or fluoride.

Stable in serum and plasma for 7 days when stored at 2…8°C.

Specimen storage and stability information provides guidance to the laboratory. Based on specific needs, each laboratory may establish alternative storage and stability information according to good laboratory practice or from alternative reference documentation.

REAGENTS

WARNING AND PRECAUTIONS

Exercise the normal precautions required for handling all laboratory reagents.

Dispose of all waste material in accordance with local guidelines.

This product contains material of animal origin. The product should be considered as potentially capable of transmitting infectious diseases.

REACTIVE INGREDIENTS

Final concentration of reactive ingredients:

Phosphate buffer (pH 6.5)	103 mmol/L
4-Aminoantipyrine	0.31 mmol/L
Phenol	5.2 mmol/L
Cholesterol esterase	≥ 0.2 kU/L (3.3 µkat/L)
Cholesterol oxidase	≥ 0.2 kU/L (3.3 µkat/L)
Peroxidase	≥ 10.0 kU/L (166.7 µkat/L)
Preservative	

The concentrations of the reactive components of the reagents shown on the kit label are the actual concentrations in the individual R1/R2 vials. The reagent composition which is shown in the Instructions For Use is the final concentration of these components in the reaction cuvette after addition of R1, Sample, and R2.

⚠ CAUTION

Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76).
To avoid the possible build-up of azide compounds, flush wastepipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations.

GHS HAZARD CLASSIFICATION

Cholesterol	WARNING	
		
	H316	Causes mild skin irritation.
	H319	Causes serious eye irritation.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P332+P313	If skin irritation occurs: Get medical advice/attention.
	P337+P313	If eye irritation persists: Get medical advice/attention.
		Ethoxylated lauryl alcohol 0.1 - 1%
		Phenol 0.2 - 0.5%
		Genapol X080 1.5 - 2.5%

SDS	Safety Data Sheet is available at beckmancoulter.com/techdocs

REAGENT PREPARATION

The reagent is ready for use and can be placed directly on board the instrument.

STORAGE AND STABILITY

The reagent is stable, unopened, up to the stated expiry date when stored at 2…8°C. Once open, reagent stored on board the instrument is stable for 90 days.

CALIBRATION

CALIBRATOR REQUIRED

System Calibrator Cat. No. 66300.

The calibrator cholesterol value is traceable to the National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 909b Level 1 (Isotope Dilution Mass Spectrometry). This method is also certified against the Centers of Disease Control Reference Method (Abell-Kendall).[4]

Recalibrate the assay when the following occur:

Change in reagent lot number or significant shift in control values;

Major preventative maintenance was performed on the analyser or a critical part was replaced.

QUALITY CONTROL

Controls Cat. No. ODC0003 and ODC0004 or other control materials with values determined by this Beckman Coulter system may be used.

Each laboratory should establish its own control frequency however good laboratory practice suggests that controls be tested each day patient samples are tested and each time calibration/blanking is performed.

The results obtained by any individual laboratory may vary from the given mean value. It is therefore recommended that each laboratory generates analyte specific control target values and intervals based on multiple runs according to their requirements. These target values should fall within the corresponding acceptable ranges given in the relevant product literature.

If any trends or sudden shifts in values are detected, review all operating parameters.

Each laboratory should establish guidelines for corrective action to be taken if controls do not recover within the specified limits.

TESTING PROCEDURE(S)

Refer to the appropriate Beckman Coulter AU analyser User Guide/Instructions For Use (IFU) for analyser-specific assay instructions for the sample type as listed in the Intended Use statement.

CALCULATIONS

The Beckman Coulter analyzers automatically compute the cholesterol concentration of each sample.

Total cholesterol levels in plasma should be corrected by multiplying the result obtained by 1.03 to be equivalent to serum levels of total cholesterol.[5]

REPORTING RESULTS

REFERENCE INTERVALS

National Cholesterol Education Program Adult Treatment Panel III recommendations:[6]

< 5.2 mmol/L (200 mg/dL)	Desirable
5.2 – 6.2 mmol/L (200 – 239 mg/dL)	Borderline High
≥ 6.2 mmol/L (240 mg/dL)	High

European Atherosclerosis Society recommendations:[7]

Cholesterol	< 5.2 mmol/L (< 200 mg/dL)	No Lipid metabolism disorder
Triglyceride	< 2.3 mmol/L (< 200 mg/dL)	
Cholesterol	5.2 – 7.8 mmol/L (200 – 300 mg/dL)	Lipid metabolism disorder if HDL-Cholesterol is < 0.9 mmol/L (< 35 mg/dL)
Cholesterol	> 7.8 mmol/L (> 300 mg/dL)	Lipid metabolism disorder.
Triglyceride	> 2.3 mmol/L (> 200mg/dL)	

National and regional guidelines for interpretation and treatment may differ from the above recommendations. Please follow the guidelines which are applicable to your population.

Expected values may vary with age, sex, sample type, diet and geographical location. Each laboratory should verify the transferability of the expected values to its own population, and if necessary determine its own reference interval according to good laboratory practice. For diagnostic purposes, results should always be assessed in conjunction with the patient's medical history, clinical examinations and other findings.

PROCEDURAL NOTES

INTERFERENCES

Results of studies conducted to evaluate the susceptibility of the method to interference were as follows:

Ascorbate :	Interference less than 10% or 0.65 mmol/L up to 3 mg/dL ascorbate
Icterus:	Interference less than 10% or 0.65 mmol/L up to 8 mg/dL or 137 µmol/L bilirubin
Haemolysis:	Interference less than 10% or 0.65 mmol/L up to 5 g/L haemoglobin
Lipemia:	Interference less than 3% or 0.195 mmol/L up to 1,000 mg/dL Intralipid

Patients treated with N-Acetyl Cysteine (NAC) for a Paracetamol overdose may generate a false low result for Cholesterol.

Venipuncture immediately after or during the administration of Metamizole (Dipyrone) may lead to falsely low results for Cholesterol. Venipuncture should be performed prior to the administration of Metamizole.

Refer to Young[8] for further information on interfering substances.

PERFORMANCE CHARACTERISTICS

PERFORMANCE CHARACTERISTICS

Data contained within this section is representative of performance on Beckman Coulter systems. Data obtained in your laboratory may differ from these values.

LINEARITY

The test is linear within a concentration range of 0.5 – 18.0 mmol/L (20 – 700 mg/dL).

SENSITIVITY

The lowest detectable level in serum on an DxC 700 AU analyzer was estimated at 0.02 mmol/L.

The lowest detectable level represents the lowest measurable level of cholesterol that can be distinguished from zero. It is calculated as the absolute mean plus three standard deviations of 20 replicates of an analyte free sample.

METHODS COMPARISON

Patient serum samples were used to compare this Cholesterol OSR6116 assay on the AU2700 against another commercially available cholesterol assay. Results of linear regression analysis were as follows:

y = 1.017x – 0.031	r = 0.996	n = 511	Sample range = 1.32 – 12.20 mmol/L

PRECISION

The following data was obtained on an AU5800 using 3 serum pools analysed over 20 days.

n = 80	Within-run		Total	
Mean mmol/L	**SD**	**CV%**	**SD**	**CV%**
2.48	0.01	0.5	0.02	0.8
6.12	0.03	0.4	0.04	0.6
13.01	0.09	0.7	0.10	0.8

ADDITIONAL INFORMATION

DxC 700 AU requires that each reagent application has a standard format of abbreviated Closed Test Name. This Closed Test Name is required to allow automated loading of the calibrator information for each application as part of the DxC 700 AU Closed System. Refer to the table below for the Closed Test Name assigned to each application for this assay.

Test Name	Description
CHO1N	Cholesterol (Serum) (NIST)
CHO2N	Cholesterol (Serum) (CDC)

Setting Sheet Footnotes
User defined
† System Calibrator Cat. No.: 66300

DxC 700 AU: † System Calibrator Cat. No: 66300 CHO1N (NIST), CHO2N (CDC).

* Values set for working in SI units (mmol/L). To work in mg/dL multiply by 38.7.

REVISION HISTORY

Revised Interferences section.

Preceding version revision history

Added new languages

REFERENCES

1. Riesen WF. Lipid Metabolism. In: Thomas L, ed. Clinical laboratory diagnostics. Use and assessment of clinical laboratory results. Frankfurt/Main: TH-Books Verlagsgesellschaft, 1998:167-169.

2. Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem 1974;20:470-475.

3. World Health Organization (2002). Use of Anticoagulants in Diagnostic Laboratory Investigations (Stability of Blood, Plasma and serum samples); WHO/DIL/LAB/99.1 Rev.2: 1-64

4. In-house data on file.

5. Current status of blood cholesterol measurement in clinical laboratories in the United States: a report from the Laboratory Standardization Panel of the National Cholesterol Education Program. Clin Chem 1988;34:193-201.

6. National cholesterol education program expert panel. Executive summary of the third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). JAMA 2001;285:2486-2497.

7. Study Group, European Atherosclerosis Society, Strategies for the prevention of coronary heart disease: A policy statement of the European Atherosclerosis Society. European Heart Journal 1987; 8, 77-88.

8. Young DS. Effects of Drugs on Clinical Laboratory Tests, AACC, 5th ed. AACC Press, 2000.

EC REP Beckman Coulter Ireland Inc., Lismeehan, O'Callaghan's Mills, Co. Clare, Ireland +(353) (0) 65 683 1100

Beckman Coulter, Inc., 250 S. Kraemer Blvd., Brea, CA 92821 U.S.A.
www.beckmancoulter.com