BECKMAN COULTER

AU/DxC AU US

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.

For Rx use only

PRINCIPLE

INTENDED USE

System reagent for the quantitative determination of Cholesterol concentrations in human serum on Beckman Coulter AU/DxC AU analyzers.

SUMMARY AND EXPLANATION

Measurements of cholesterol are used primarily in the diagnosis and treatment of disorders involving excess cholesterol in the blood, and lipid and lipoprotein metabolism disorders.

Total serum cholesterol analysis has proven useful in the diagnosis of hyperlipoproteinemia, atherosclerosis, hepatic and thyroid diseases.[1] Total and HDL cholesterols, in conjunction with a triglyceride determination, provide valuable information for the prediction of coronary heart disease.[2]

METHODOLOGY

Assays of total cholesterol in saponified serum extracts using “cholesterol dehydrogenase” was begun by Flegg[3] and Richmond[4]. Previously, Hernandez and Chaikoff [5] and Hyun et al.[6] had isolated a cholesterol ester hydrolase which was effective in producing free cholesterol from cholesterol esters. Finally, in 1974, Allain et al. [7] and Rieschlau et al. [8] were able to combine the esterase and oxidase into a single enzymatic reagent for the determination of total cholesterol; this is the basis for the Cholesterol method.

Cholesterol esters in serum are hydrolyzed by cholesterol esterase (CHE). The free cholesterol produced is oxidized by cholesterol oxidase (CHO) to cholest-4-en-3-one with the simultaneous production of hydrogen peroxide (H_20_2), which oxidatively couples with 4-aminoantipyrine and phenol in the presence of peroxidase to yield a chromophore.

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

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

$$\text{Cholesterol + } O_2 \xrightarrow{\text{CHO}} \text{Cholest-4-en-3-one + } H_2O_2$$

$$2\ H_2O_2 + \text{4-aminoantipyrine + phenol} \xrightarrow{\text{Peroxidase}} \text{Red dye + 4 } H_2O_2$$

SPECIMEN

SPECIMEN STORAGE AND STABILITY

Total cholesterol in serum/plasma has been reported to be stable for at least 7 days when stored at 4 - 8°C and up to 3 months when stored at ≤ -20°C.[9]

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.

Additional handling conditions as designated by this laboratory:

SPECIMEN COLLECTION AND PREPARATION

Serum samples and K2/K3 EDTA or Li/Na Heparinized plasma, free from hemolysis, are the recommended specimens. Separate serum from blood cells as soon as possible. Plasma is not recommended using anticoagulants such as oxalate, citrate or fluoride.[10] Total Cholesterol levels in EDTA plasma should be corrected by multiplying the result obtained by 1.03 to be equivalent to serum levels of Total Cholesterol.[11]

Additional instructions for patient sample preparation as designated by this laboratory:

Additional type conditions as designated by this laboratory:

REAGENTS

CONTENTS

Cholesterol Reagent.

Reagent storage location in this laboratory:

WARNING AND PRECAUTIONS

1. Exercise the normal precautions required for handling all laboratory reagents.
2. Dispose of all waste material in accordance with local guidelines.
3. 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	
Cholesterol Esterase (Candida/Pancreatic)	≥ 0.2 kU/L	(3.3 µkat/L)
4-Aminoantipyrine	0.31 mmol/L	
Cholesterol Oxidase (Brevibacterium)	≥ 0.2 kU/L	(3.3 µkat/L)
Phenol	5.2 mmol/L	
Peroxidase (Horseradish)	≥ 10.0 kU/L	(166.7 µkat/L)
Also contains preservatives		

⚠ 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

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

Chemistry Calibrator (Cat # DR0070)

Storage location of the Calibrator in this laboratory:

EQUIPMENT AND MATERIALS

For use on the AU480, AU680, AU5800, DxC 500 AU, DxC 500i and DxC 700 AU Beckman Coulter Analyzers.

OSR6616 for use on the AU5800 systems only.

Storage location of test tubes or sample cups in this laboratory:

REAGENT PREPARATION

The Cholesterol Reagents are ready for use. No preparation is required.

STORAGE AND STABILITY

1. The unopened reagent is stable until the expiration date printed on the label when stored at 2 - 8°C.
2. Opened reagent is stable for 90 days when stored in the refrigerated compartment of the analyzer.

INDICATIONS OF DETERIORATION

Visible signs of microbial growth, turbidity or precipitate, or any change in color in the Cholesterol reagent may indicate degradation and warrant discontinuance of use.

The reagent is normally pink in color.

Additional storage requirements as designated by this laboratory:

STABILITY OF FINAL REACTION MIXTURE

The Beckman Coulter AU/DxC AU analyzer automatically computes every determination at the same time interval.

CALIBRATION

CALIBRATOR REQUIRED

The frequency of calibration is every 30 days. Calibration of this cholesterol procedure is accomplished by use of the Chemistry Calibrator (Cat # DR0070). For Traceability information refer to the calibrator instructions for use.

Recalibration of this test is required when any of these conditions exist:

1. A reagent lot number has changed or there is an observed shift in control values.
2. Major preventative maintenance was performed on the analyzer.
3. A critical part was replaced.

QUALITY CONTROL

During operation of the Beckman Coulter AU/DxC AU analyzer, at least two levels of an appropriate quality control material should be tested a minimum of once a day. In addition, controls should be performed after calibration with each new lot of reagent, and after specific maintenance or troubleshooting steps described in the appropriate Beckman Coulter AU/DxC AU analyzer Instructions For Use (IFU) and Reference Manual. Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure.

Location of controls used at this laboratory.

CONTROL NAME	SAMPLE TYPE	STORAGE

TESTING PROCEDURE(S)

A complete list of test parameters and operational procedures are provided in the relevant AU/DxC AU analyzer IFU and Reference Manual.

RESULTS INTERPRETATION

The default unit of measure is mg/dL, for conversion to SI units (mmol/L) the result is multiplied by 0.0259.

REPORTING RESULTS

EXPECTED RESULTS

Total Cholesterol	Risk Classification[12]
< 200 mg/dL	Desirable
200 - 239 mg/dL	Borderline High
> 240 mg/dL	High

Reference Intervals shown above were taken from the literature. 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.

REFERENCE INTERVALS

The following data was obtained using the Cholesterol Reagent on Beckman Coulter AU analyzers according to established procedures. Results obtained in individual laboratories may differ.

Expected reference ranges in this laboratory:

INTERVALS	SAMPLE TYPE	UNITS (mg/dL)

Additional reporting information as designated by this laboratory:

PROCEDURAL NOTES

INTERFERENCES

Results of studies[13] show that the following substances interfere with this cholesterol assay.

The criteria for no significant interference is recovery within 10% or 25.2 mg/dL of the initial value.

Ascorbate:	No significant interference up to 3 mg/dL Ascorbate
Bilirubin:	No significant interference up to 8 mg/dL Bilirubin
Hemolysis:	No significant interference up to 500 mg/dL Hemolysate
Lipemia:	No significant interference up to 1,000 mg/dL Intralipid*

*Intralipid, manufactured by KabiVitrium Inc., is a 20% IV fat emulsion used to emulate extremely turbid samples.

Patients treated with N-Acetyl Cysteine (NAC) for a acetaminophen 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.

The information presented is based on results from Beckman Coulter studies and is current at the date of publication. Beckman Coulter Inc., makes no representation about the completeness or accuracy of results generated by future studies. Further information on interfering substances is available.[14]

Laboratory specific procedure notes:

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.

SENSITIVITY

Typical change in absorbance for 1 mg/dL of Cholesterol is 1.1 mAbsorbance.

DYNAMIC RANGE / ANALYTICAL MEASURING RANGE

The Cholesterol procedure is linear from 25 to 700 mg/dL. Samples exceeding the upper limit of linearity should be diluted and repeated. The sample may be diluted, repeated and multiplied by the dilution factor automatically utilizing the AUTO REPEAT RUN.

METHODS COMPARISON

Reference[15]

Patient serum samples were evaluated in method comparison studies.

Results of Deming regression analysis were as follows:

Y Method	DxC 700 AU
X Method	AU5800
Slope	1.002
Intercept	-1.650
Correlation Coeff. (r)	1.000
No. of Samples (n)	120
Range (mg/dL)	26 - 660

PRECISION

Reference[15]

Estimates of precision, based on CLSI recommendations,[16] are consistent with typical performance. The within run and total precision is less than 3% CV. Assays of control sera were performed and this data reduced following CLSI guidelines above.

N = 80	Within-run		Total	
Mean mg/dL	SD	CV%	SD	CV%
89.4	0.5	0.5	0.6	0.7
221.5	0.9	0.4	1.4	0.6
470.5	2.6	0.6	3.4	0.7

ADDITIONAL INFORMATION

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

Test Name	Description
CHO1U	Cholesterol (Serum)

Refer to the Beckman Coulter Chemistry Systems Reagent Guide (BAGUIDE) for specific chemistry information for the AU/DxC AU clinical chemistry systems and guidance on symbols used on all AU/DxC AU product labelling.

Setting Sheet Footnotes

User defined

Lot or Lot + Bottle

† Beckman Coulter System Calibrator Cat No.: DR0070

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

REVISION HISTORY

Updated Specimen Section

Updated REPORTING RESULTS section

Revised Interferences section.

Updated precision section

Add DxC 500i instrument to IFU

Updated References section

Preceding version revision history

Revised Methodology section

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