



AU®US

# Instructions For Use

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# CHOL Cholesterol

**REF**

OSR6116 4 x 22.5 mL R1  
OSR6216 4 x 45 mL R1  
OSR6516 4 x 107 mL R1  
OSR6616 4 x 173 mL R1

**For *in vitro* diagnostic use only.**

## ANNUAL REVIEW

Reviewed by	Date	Reviewed by	Date

## PRINCIPLE

### INTENDED USE

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

OSR6516 for use on the AU680, AU2700 and AU5400 systems only

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

### 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.<sup>1</sup> Total and HDL cholesterol, in conjunction with a triglyceride determination, provide valuable information for the prediction of coronary heart disease.<sup>2</sup>

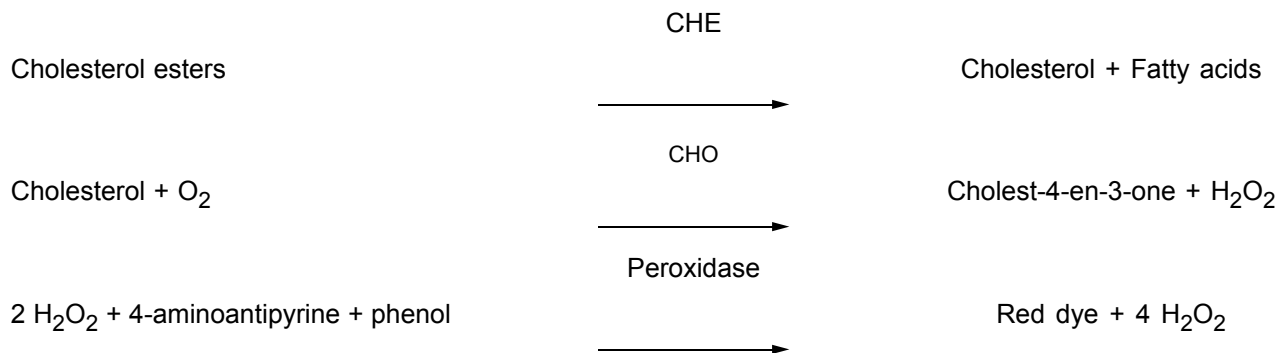
### METHODOLOGY

Assays of total cholesterol in saponified serum extracts using “cholesterol dehydrogenase” was begun by Flegg<sup>3</sup> and Richmond<sup>4</sup>. Previously, Hernandez and Chaikoff<sup>5</sup> and Hyun et al.<sup>6</sup> had isolated a cholesterol ester hydrolase which was effective in producing free cholesterol from cholesterol esters. Finally, in 1974, Allain et al.<sup>7</sup> and Rieschlau et al.<sup>8</sup> 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.

**The Cholesterol reagent has been certified to meet the National Cholesterol Education Program’s (NCEP) performance criteria for accuracy.**

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<sub>2</sub>O<sub>2</sub>), 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.



## SPECIMEN

### SPECIMEN STORAGE AND STABILITY

Total cholesterol in serum has been reported to be stable for at least 7 days when stored at 2 - 8°C, up to 3 months when stored at ≤ -20°C and years at -70°C.<sup>9</sup>

### SPECIMEN COLLECTION AND PREPARATION

Serum samples and EDTA or 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.<sup>9</sup> 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.<sup>10</sup>

## REAGENTS

### CONTENTS

Cholesterol Reagent.

### WARNING AND PRECAUTIONS

1. **WARNING! POISON!** Contains phenol which may be fatal if swallowed. Avoid contact with eyes, skin and mucous membranes. In case of external contact, immediately rinse affected area with large amounts of water. Obtain medical attention immediately for eye contact or ingestion.
2. Contains sodium azide as a preservative which may react with lead joints in copper plumbing to form explosive compounds. Even though the reagent contains minute quantities of sodium azide, drains should be well flushed with water when discarding the reagent.

### 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.

EUH208

May produce an allergic reaction.

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%

Peroxidase < 0.1%

Phenol 0.2 - 0.5%

Genapol X080 1.5 - 2.5%

### MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

Chemistry Calibrator (Cat # DR0070)

### EQUIPMENT AND MATERIALS

For AU400/400<sup>e</sup>/480, AU600/640/640<sup>e</sup>/680 and AU2700/5400/AU5800 Beckman Coulter Analyzers.

### 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.

## STABILITY OF FINAL REACTION MIXTURE

The Beckman Coulter 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 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 User's Guide. Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure.

## TESTING PROCEDURE(S)

A complete list of test parameters and operational procedure can be found in the User's Guide appropriate to the analyzer.

## RESULTS INTERPRETATION

Results are automatically printed out for each sample in mg/dL at 37°C.

For SI Units (mmol/L) the results must be multiplied by 0.0259.

## REPORTING RESULTS

### EXPECTED RESULTS

<u>Total Cholesterol</u>	<u>Risk Classification</u> <sup>11</sup>
< 200 mg/dL	Desirable
200 - 239 mg/dL	Borderline High
> 240 mg/dL	High
Reference Range <sup>12</sup>	136 - 290 mg/dL

Expected values may vary with age, sex, diet and geographical location. Each laboratory should determine its own expected values as dictated by good laboratory practice.

## 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.

## PROCEDURAL NOTES

### INTERFERENCES

Results of studies<sup>13</sup> show that the following substances interfere with this cholesterol assay.

The criteria for no significant interference is recovery within 10% 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 1000 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.

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. For further information on interfering substances, refer to Young<sup>14</sup> for a compilation of reported interferences with this test.

## PERFORMANCE CHARACTERISTICS

### DYNAMIC 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.

### SENSITIVITY

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

### METHODS COMPARISON

Reference<sup>15</sup>

Patient samples were used to compare this Cholesterol Reagent. The table below demonstrates representative performance on the AU analyzers.

Y Method	AU640
X Method	AU600
Slope	0.988
Intercept	+ 0.2
Correlation Coeff. (r)	1.000
No. of Samples (n)	183
Range (mg/dL)	33-448

## PRECISION

Reference <sup>15</sup>

Estimates of precision, based on CLSI recommendations,<sup>16</sup> 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 = 100	Within-run		Total	
	SD	CV%	SD	CV%
Mean mg/dL				
115.5	0.5	0.5	1.3	1.1
252.2	0.9	0.3	2.7	1.1

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