CHOLESTEROL

CHOD-PAP

Intended use:
Enzymatic in vitro test for the quantitative determination of cholesterol in human serum and plasma.

Summary:
Cholesterol is a steroid with a secondary hydroxyl group in the C_3 position. It is syn- thesized in many types of tissue, but particularly in the liver and intestinal wall. Approximately three quarters of cholesterol is newly synthesized and a quarter originates from dietary intake. Cholesterol assays are used for screening for athero- sclerotic risk and in the diagnosis and treatment of disorders involving elevated cholesterol levels as well as lipid and lipoprotein metabolic disorders. Cholesterol analysis was first reported by Liebermann in 1885 followed by Burchard in 1889. In the Liebermann-Burchard reaction, cholesterol forms a blue-green dye from polymeric unsaturated carbohydrates in an acetic acid/acetic anhydride/ concentrated sulfuric acid medium. The Abell and Kendall method is specific for cholesterol, but is technically complex and requires the use of corrosive reagents. In 1974, Roeschlau and Allain described the first fully enzymatic method. This method is based on the determination of A^4 cholestenone after enzymatic cleavage of the cholesterol ester by cholesterol esterase, conversion of cholesterol by cholesterol oxidase and subsequent measurement by the Trinder reaction of the hydrogen peroxide formed. Optimization of ester cleavage (>99.5%) allows standardization using primary and secondary standards and a direct comparison with the CDC and NIST reference methods. The Analyticon cholesterol assay meets the 1992 National Institutes of Health (NIH) goal of less than or equal to 3% for both precision and bias.

Test principle:

Cholesterol ester + H_2O —(Cholesterol esterase)→ Cholesterol + fatty acids

Cholesterol esters are ceaved by the action of choesterol esterase to yield free choesterol and fatty acids

Cholesterol + O_2 —(Cholesterol oxidase)→ Cholesten-3-on + H_2O_2

$2H_2O_2$ + Phenol + 4-Aminoantipyrine —(Peroxidase)→ Quinoneimine dye + 4 H_2O

Cholesterol is converted by oxygen with the aid of cholesterol oxidase to A^4-Cholestenone and hydrogen peroxide. Hydrogen peroxide created forms a red dyestuff by reacting with 4-aminoantipyrine and phenol under the catalytic action of peroxidase. The color intensity is directly proportional to the concentration of cholesterol and can be determined photometrically.

Reagent concentration:

R1:	
Pipes buffer, pH 6.9 Phenol	90 mmol/l
Cholesterol oxidase	26 mmol/l
Cholesterol esterase	200 U/l
Peroxidase	1250 U/l
4-Aminoantipyrine	0,4 mmol/l

Preparation and stability:
Reagent and standard are ready for use.
The unopened kit components: Up to expiry date at +2°C to +8°C
Onboard stability: R1: 28 days

Specimen:
Collect serum using standard sampling tubes.
Heparinized or EDTA plasma. Do not use citrate, oxalate or fluoride-plasma. Stability: 5 - 7 days at +2°C to +8°C
3 months at -20°C
Fasting and nonfasting samples can be used.

Limitations - interference:
Criterion: Recovery within ±10% of initial value.

Icterus: No significant interference up to an index I of 8 (approximate bilirubin concentration: 8 mg/dl).
Hemolysis: No significant interference up to an index H of 450 (approximate hemoglobin concentration: 450 mg/dl).

Testing procedure:

Applications for automated systems are available on request.

Materials provided
· Working solutions as described above *Additional materials required*
· Calibrators and controls as indicated below
· 0.9% NaCl

Manual procedure		
Wavelength:	540 nm	
Temperature:	+37°C	
Cuvette:	1 cm light path	
Zero adjustment:	Reagent blank	
	Blank	Sample / Calibrator
Sample / Calibrator	-	10 µl
R1	1000 µl	1000 µl
Mix and incubate 5 minutes. Read the absorbance against blank within 30 minutes.		
Calculation: $\frac{AA\ sample}{AA\ Calibrator}$ x Calibrator conc. = Cholesterol in mg/dl		

Measuring/reportable range:
30-800 mg/dl
Determine samples having higher activities via the rerun function. On instruments without rerun function, manually dilute the samples with 0.9% NaCl-solution or distilled/deionized water (e.g. 1 + 2). Multiply the result by the appropriate dilution factor (e.g. factor 3).

Expected values:

Clinical Interpretation according to the recommendations of the European atherosclerosis Society.		
Cholesterol Triglycerides	below 200 mg/dl	No
Cholesterol	200 - 300 mg/dl	yes if HDL-Cholesterol below 33 mg/dl
Cholesterol Triglycerides	above 300 mg/dl	Yes

Recommendations of the NCEP Adult Treatment Panel for the following risk-cutoff thresholds for the US American population
Desirable cholesterol level: <200 mg/dl
Borderline high cholesterol: 200-239 mg/dl
High cholesterol: >240 mg/dl
Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range. For diagnostic purposes the cholesterol results should always be assayed in conjunction with the patient's medical history, clinical examinations and other findings. At least two measurements of cholesterol on separate occasions should be made before any medical decision is made, since a single point total cholesterol measurement may not represent a patient's usual cholesterol concentration.

Analytical sensitivity (lower detection limit)
Detection limit: 30 mg/dl
The lower detection limit represents the lowest measurable cholesterol concentration that can be distinguished from zero.

Method comparison:
A comparison of the BIOANALYTIC CHOL (y) with a commercial obtainable assay (x) gave following result:y = 1.006 x + 0.258; r = 0.999

Quality Control:
Control Serum:

BIOCON N	5 x 5 ml	# B10814
BIOCON P	5 x 5 ml	# B10817

The control intervals and limits must be adapted to the individual laboratory and country-specific requirements. Values obtained should fall within established limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

Calibration:
S1: 0.9% NaCl
S2: BIOCAL H 5 x 3 ml # B11895

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Calibration frequency:

A two-point-calibration is recommended in case of:
1-change of lot
2- quality control requirements

Imprecision:

Reproducibility was determined using controls. The following results were obtained:

	Between day		
Sample	Mean mg/dl	SD mg/dl	% CV
Control serum 1	124	1.71	1.38
Control serum 2	162	3.13	1.93
Control serum 3	186	1.97	1.06

Literature:

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Order information (Cat No.) :

CC380	AB380	B25080	B30080	B33081	B37081
SH380	BCHOL250	B25081	B30081	B34080	B42080
CR380	B21080	B27080	B31080	B35080	B80080
OL380	B21081	B27081	B32080	B36080	B80081
KL380	B22080	B28080	B32081	B36081	B80082
BCHOL500	B24080	B28081	B33080	B37080	

Manufacturer

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SYMBOLS

IVD	for in vitro diagnostic use only
LOT	lot of manufacturing
REF	code number
	storage at temperature interval
	expiration date (year/month)
	warning, read enclosed documents
	Read the directions