CHOLESTEROL

Diagnostic reagent for determination of Cholesterol concentration.
Liquid. Monoreagent. Store at +2/+8°C. For in Vitro Diagnostic Use (IVD). **Do not freeze.**

Ref No	Pack
MH-082	200 mL
MH-083	120 mL

Changes made in the instructions for use are marked as grey.

INTENDED USE

The test is applied for the quantitative determination of cholesterol in serum and plasma.

GENERAL INFORMATION

Cholesterol is a sterol compound that is found in all animal tissues and serves various important physiological functions, including being a substrate for the synthesis of bile acids and steroid hormones; it is an important component in cell membranes. Because cholesterol appears to be involved in atherosclerosis, cholesterol measurement is one of the most common laboratory tests used today.[1]

The history of this organic compound dates back to the 19th century. Liebermann first described the color reaction of sulfuric acid with a cholesterol solution in acetic anhydride in 1885.[2] Four years later, Burchard reported that a more intense blue-green color was produced when acetic anhydride and sulfuric acid were added to a solution of cholesterol in chloroform.[3] These discoveries paved the way for the development of many colorimetric methods for cholesterol measurement.

Total cholesterol (TC) is one of the four parameters recommended for the diagnosis of dyslipidemia. Studies have so far used TC and LDL-C levels for risk calculation and evaluation of response to treatment. In addition, there is a lot of scientific evidence that reducing TC and LDL-C levels reduces mortality. Although a relationship between postprandial triglyceride (TG) levels and increased cardiovascular risk has recently been reported, the idea that treatment of elevated TG levels reduces cardiovascular risk in clinical practice is controversial. Therefore, TC and LDL-C are the primary targets in the treatment of dyslipidemia.[4]

In the Systematic Coronary Risk Evaluation (SCORE) calculation system developed by the European Society of Cardiology, in addition to age, gender, smoking, systolic blood pressure, TC values are among the risk factors.

According to these parameters, the 10-year risk of fatal atherosclerotic cardiovascular disease (ASCVD) is calculated in populations at high risk of cardiovascular disease.[5]

Hypolipidemia can be found in 3% of the general population and in 6% of inpatients, and in some cases it can be a marker of upcoming serious diseases and sometimes the cause of them.

Hypolipidemia is also referred to as "hypocholesterolemia" because it refers to low plasma LDL-C, TC and apolipoprotein B (apo-B) levels. It is accepted as plasma cholesterol levels below 120-150 mg/dL and LDL-C levels below 50-80 mg/dL or values below the 5th percentile in the population. It may have primary (congenital) and secondary (acquired) causes.

Abetalipoproteinemia, hypobetalipoproteinemia and chylomicron retention disease are examples of primary causes, while malnutrition, malabsorption, hyperthyroidism, liver failure, sepsis, burns, some types of anemia, chronic diseases, cancers and lipid-lowering drugs are examples of secondary causes.[23]

TEST PRINCIPLE

Colorimetric measurement

All cholesterol esters in serum are enzymatically hydrolyzed by cholesterol esterase to cholesterol and free fatty acids. In the presence of oxygen, free cholesterol is hydrolyzed to cholest-4-ene-3-one and hydrogen peroxide (H_2O_2) by cholesterol oxidase. H_2O_2 reacts with p-chlorophenol and 4-aminoantipyrine in the presence of peroxidase to form the chromophore quinonimine dye. The intensity of the color formed is proportional to the cholesterol concentration and can be measured photometrically between 480-520 nm wavelengths.

REAGENT COMPONENTS

Sodium cholate	: ≤ 8.3 mmol/L
Cholesterol esterase	: ≥ 400 U/L
Cholesterol oxidase	: ≥ 200 U/L
Peroxidase	: ≥ 500 U/L
4-aminoantipyrine	: ≤ 0.8 mmol/L
4-chlorophenol	: ≤ 2.4 mmol/L
Good's buffer	

REAGENT PREPARATION

Reagent is ready for use.

REAGENT STABILITY AND STORAGE

Reagents are stable at +2/+8°C till the expiration date stated on the label which is only for closed vials.

Once opened vials are stable for 60 days at +2/+8°C in optimum conditions. On board stability is strongly related to auto analyzers' cooling specification and carry-over values.

Reagent stability and storage data have been verified by using Clinical and Laboratory Standards Institute (CLSI) EP25-A protocol.[6]

SAMPLE REQUIREMENTS

Serum and plasma can be used and are collected according to the standard procedures. For plasma, sample collection tubes with Li heparin or K_2-EDTA should be preferred. Multiple sample freezing and thawing should be avoided.

Cholesterol activity stability in serum and plasma[24,25]:

7 days at +2/+8ºC
7 days at +20/+25ºC
3 months at -20°C

Annotation:

- If phlebotomy is performed properly, plasma or serum is usually suitable for TC measurements. However, since plasma and serum cholesterol values differ by 3% to 5%, the NCEP Laboratory Standardization Panel recommends that if plasma is used, cholesterol values should be multiplied by 1.03 to make the values equivalent to serum values.[7]
- Plasma is usually preferred when analyzing lipids and lipoproteins chemically. If plasma is chosen, the recommended anticoagulant is solid EDTA (1 mg/mL blood) and blood cells should be separated within a maximum of 2 hours.[8]
- It is well known that when a standing person changes to the supine position, plasma volume increases and concentrations of non-diffusible plasma components decrease as a result of water redistribution between vascular and extravascular compartments. A significant reduction in total plasma cholesterol has been measured after 5 minutes and reductions of 10% to 15% have been recorded 20 minutes after moving to a horizontal position.
  The effect on cholesterol concentration when the person moves from a standing to a sitting position is also significant, but somewhat smaller (about 6% after 10 to 20 minutes in the sitting position).[8]
- If a tourniquet is used, applying a tourniquet for 2 minutes increases TC concentrations by 2% to 5%; increasing this to 5 minutes causes an average elevation of 10% to 15%. Changes after holding the tourniquet for 30 to 60 seconds are generally insignificant.[8]

CALIBRATION AND QUALITY CONTROL

Calibration: The assay requires the use of an Arcal Auto Calibrator

Arcal Auto Calibrator-Lyophilized
Ref.No: VT-003

Calibration stability is 60 days. Calibration stability depends on the application characteristics and cooling capacity of the autoanalyzer used.

Cholesterol was standardised according to Abell/Kendall and isotope dilution/mass spectrometry.

Control: Commercially available control material with established values determined by this method can be used. We recommend:

Arcon N Level 1 Control- Lyophilized
Ref.No: VT-001

Arcon P Level 2 Control- Lyophilized
Ref.No: VT-002

At least two level controls must be run once in every 24 hours. Each laboratory should determine its own quality control scheme and procedures. If quality control results are not within acceptable limits, calibration is required.

REFERENCE INTERVALS / MEDICAL DECISION LEVELS

According to the Adult Treatment Panel III (ATP III) guideline, an updated high blood cholesterol treatment guideline published by the National Cholesterol Education Program (NCEP), a TC value <200 mg/dL is defined as normal; values between 200-239 mg/dL are defined as borderline high; ≥240 mg/dL is defined as high.[9]

Normal	: < 200 mg/dL
Borderline high	: 200- 239 mg/dL
High	: ≥ 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.

Reference interval has been verified by using CLSI EP28-A3c protocol.[10]

Unit Conversion:
mg/dL x 0.0259 = mmol/L

PERFORMANCE CHARACTERISTICS

Measuring Interval

According to CLSI EP34-ED1:2018, "Measuring Interval" refers to the interval where the analyte concentration is measured with intended accuracy in terms of medical and laboratory requirements without dilution, concentrating or any kind of pre-treatment that is between the analyte's lower limit of quantitation (LLoQ) and upper limit of quantitation (ULoQ).[11]

The determined analytic measuring interval for Cholesterol is 7-700 mg/dL.

Detection Capability

Limit of Detection (LoD): 5 mg/dL

Limit of Quantitation (LoQ): 7 mg/dL

Note: LoQ values are based on Coefficient of Variation Percentage (CV) ≤ 20%.

LoD and LoQ values have been verified by using CLSI EP17-A2:2012 protocol.[12]

Linearity

This method shows measurement linearity in the activities up to 700 mg/dL.Autoanaylzer's auto-dilution system can be used if the concentrations have higher values. See device manual for further information.

For the manual dilution procedure, dilute the sample 1:5 using 0.90% isotonic. After this process, multiply the result of the reworked sample by the dilution factor. Do not report the sample result after dilution if it is marked as lower than the linear lower limit. Rerun with a suitable dilution.

Linearity Studies data have been verified by using CLSI EP06-A:2003 protocol.[13]

Precision

Running system has been developed according to 20x2x2 "The Single Site" protocol. Repeatability and Within-Laboratory Precision/Within-Device values have been obtained according to the running results.

According to the protocol in use, 2 separate runs per day have been made for 20 days (no obligation for being consecutive days). This protocol has been applied to each low and high samples separately and 80 results have been obtained for each one. Statistically, the results have been obtained using 2-factor Nested-ANOVA model.[14]
Repeatability (Within Run) and Repeatability (Day to Day) SD (standard deviation) and CV% values of Cholesterol have been given in the table 1 and 2 respectively.

Table 1. Cholesterol Repeatability (Within Run) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
184 mg/dL	2.60	1.42	80
267 mg/dL	2.76	1.03	80

Note: This working system has been named "Within-Run Precision" in the previous CLSI - EP05-A2 manual.[15]

Table 2. Cholesterol Repeatability (Day to Day) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
184 mg/dL	3.39	1.84	80
267 mg/dL	6.68	2.50	80

Note: This working system has been named "Total Precision" in the previous CLSI - EP05-A2 manual.[15]

Method Comparison

As a result of the statistical evaluation of the method comparison data:

Passing-Bablock equation:[16]
y= 0.979x + 1,71 mg/dL
r=0.995

Interference

Endogenous interferant and analyte concentrations that have been used in the Cholesterol scanning tests has been determined according to "CLSI EP37-ED1:2018" and "CLSI EP07-ED3:2018" manuals.[17,18]

The total acceptable error rate, which is going to be used to detect whether the observed differential value obtained from Cholesterol interference scanning test is appropriate, is determined as ±10%.[19]

In Cholesterol test results, no significant interaction has been observed in the determined endogenous interferant and analyte concentrations or between interferants and analyte.

Interferant-Concentration	Cholesterol Target (mg/dL)	N*	Observed Recovery %
Hemoglobin 630 mg/dL	169,3	3	109
Bilirubin 12,5 mg/dL	180,3	3	91
Lipemia 433,4 mg/dL	175,6	3	108

** Total acceptable error rate determined as interference limit and repeatability (within run) pre-detected for the related method were used for the calculations of how many times the control and test samples prepared as a serum pool are going to be run repetitively. In the calculations, the accepted error rate for type 1 (α error) was 5% and for type 2 (β error) was 10% (90% power).*[18]

Annotation:

- Some sources have reported that bilirubin may have an inhibitory effect in enzymatic assays of serum cholesterol performed by interaction of peroxidase with 4-aminoantipyrine and phenol.[20,21] Miner-Williams reported that some surfactants may inhibit cholesterol oxidase activity.[22]

It should be noted that endogenous interferants, as well as various medicines and metabolites, anticoagulants (e.g. Heparin, EDTA, citrate, oxalate) and preservatives (e.g.

sodium floride, iodoacetate, hydrochloride acide) such as additives, materials that may contact with samples during collection and processing (serum separator devices, sample collection containers and contents, catheters, catheter wash solutions, skin disinfectants, hand cleaners and lotions, glass washing detergents, powder gloves), dietary substances known to affect some specific tests (caffeine, beta-carotene, poppy seeds, etc.), or some substances present in a sample that cause foreign proteins (heterophilic antibodies, etc.), autoimmune response (autoantibodies, etc.), or due to malignancy (for example, interference by paraproteins with phosphate testing and indirect ion selective electrode methods) may show some negative effects that will cause various attempts and some misjudgements.[18]

These performance characteristics have been obtained using an autoanalyzer. Results may vary slightly when using different equipment or manual procedures.

WARNINGS AND PRECAUTIONS

IVD: For in Vitro Diagnostic use only.
Do not use expired reagents.
Reagents with two different lot numbers should not be interchanged.
For professional use.
Follow Good Laboratory Practice (GLP) guidelines.

CAUTION: Human source samples are processed with this product. All human source samples must be treated as potentially infectious materials and must be handled in accordance with OSHA (Occupational Safety and Health Administration) standards.

Danger

EUH032	:Releases a very toxic gas if contacts with acid.
H317	:May cause allergic skin reaction.

Precaution

P280	:Use protective gloves / clothes / glasses / mask.
P264	:Wash your hands properly after using.
P272	:Contaminated work clothes should not be allowed to be used outside of the workplace.

Intervention

P302+P352	:Wash with plenty of water and soap if it contacts with skin.
P333+P313	:Seek medical help if it irritates your skin or develops rash.
P362+P364	:Remove contaminated clothes and wash properly before using.

Disposal

P501	:Dispose the vials and contents according to the local regulations.

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SYMBOLS

IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
R1	Reagent 1
GTIN	Global Trade Item Number
REF	Reference Number
GLP	Good Laboratory Practices
FOR USE WITH	Identifies Products to Be Used Together
PRODUCT OF TURKEY	Product of Turkey
	Manufacturer
	Expiration Date
	Temperature Limits
	Consult Instructions for Use
	Caution
	Number of Tests

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