DIRECT HDL CHOLESTEROL

(D-HDL-C)

Diagnostic reagent for determination of HDL (High Density Lipoprotein) concentration.

Liquid. Dual reagent. Store at +2/+8°C. For In Vitro Diagnostic use. **Do not freeze**.

Ref No	Package	Ref No	Package	Ref No	Package	Ref No	Package
At220	256,8 mL	HN340	360 mL	LM162	160 mL	PL2101	136 mL
BB055	208 mL	HN341	240 mL	LM163	320 mL	RC220	57 mL
BYH220	720 mL	KHD21	360 mL	LM164	240 mL	RC223	46 mL
BYH221	540 mL	LB230	240 mL	LM165	160 mL	RDA220	360 mL
BZ2055	360 mL	LB231	240 mL	MDD20	300 mL	RDA221	180 mL
DMHD20	307,5 mL	LHD20	720 mL	MDD21	180 mL	RDA222	240 mL
D2230	400 mL	LHD21	360 mL	MHD20	480 mL	S2100	400 mL
D2231	200 mL	LHD22	240 mL	MHD21	200 mL	S2102	100 mL
ERD20	160 mL	LHD23	320 mL	M3D20	260 mL	TBHD20	240 mL
ERD21	240 mL	LM160	320 mL	M3D21	220 mL	TBHD21	160 mL
HD2211N	400 mL	LM161	240 mL	M3D22	80 mL	8AH220	720 mL
HD2213N	100 mL			M4D20	480 mL	8AH221	540 mL
HD2215N	400 mL			M4D21	200 mL	MH-092	80 mL

INTENDED USE

The Direct HDL Cholesterol (D HDL-C) test is an in vitro assay used in clinical laboratories for the quantitative determination of High Density Lipoprotein Cholesterol (HDL-C) concentration in human serum and plasma using analyzers.

GENERAL INFORMATION

HDL-C is synthesized in the liver and small intestine and has the highest density (1.063-1.210 g/ml) of the four major lipoproteins. HDL-C is a molecule rich in protein and phospholipids and poor in carbohydrate content. ApoA-I is the main protein in HDL. Apo B-100, Apo B-48 and other apolipoproteins except Apo(a) are present in its structure in certain proportions. It also contains the enzyme lecithin cholesterol acyl transferase (LCAT), which catalyzes the conversion of cholesterol into the more hydrophobic cholesteryl ester structure, thus facilitating the transport of cholesterol from peripheral tissues to the liver via HDL.

HDL-C transports cholesterol stored in extrahepatic tissues, including foam cells in newly formed plaques, from peripheral cells to the liver. Through this mechanism, called the reverse cholesterol transfer pathway, in which some transporter molecules such as ABCA1 (interacts with Apo A-I in HDL-C) and ABCG1 (interacts with mature HDL molecule) are also involved, HDL exerts a protective effect against atherosclerosis by reducing the cholesterol content of foam cells. Clinical and epidemiologic studies have reported that high blood HDL concentrations have a protective effect against Coronary Heart Disease (CHD). In rare familial HDL deficiency and Tangier disease, the function of these transporter molecules in the regulation of plasma HDL levels is impaired.[1]

The risk of developing CHD is increased in some lipoprotein metabolism disorders, such as hypoalphalipoproteinemia, which results in low HDL levels due to various genetic defects. Mutations and deletions in apo A-I related genes or low HDL levels due to LCAT deficiency may cause pathologies such as cloudy cornea due to infiltration of lipids in the cornea or glomerulosclerosis as a result of the formation of abnormal lipoprotein particles and their deposition in the glomeruli.[2]

In clinical studies, low HDL-C levels have been associated with an increased risk of cardiovascular disease (CVD)[1] and reported as a critical risk factor for predicting 10-year CVD risk [2]

TEST PRINCIPLE

Homogeneous Enzymatic Colorimetric Method

HDL-C measurement is based on the determination of HDL-related cholesterol concentration. Basically, the HDL-C concentration is determined by measuring total cholesterol after blocking non-HDL lipoproteins so that they do not react enzymatically. Based on the direct measurement of HDL-C, this diagnostic test kit does not require sample pre-treatment.

In this method, dextran sulfate, a polyanion, reacts with positively charged groups on non-HDL lipoproteins such as LDL, VLDL and chylomicrons to form a water-soluble complex. This interaction is activated in the presence of a divergent cation such as magnesium. This complex prevents cholesterol esterase and cholesterol oxidase enzymes from reacting with non-HDL lipoproteins.

HDL-C is then solubilized with a special detergent and the ester groups are removed through a reaction catalyzed by PEG-Cholesterol Esterase, resulting in free HDL-C. This

free HDL-C undergoes oxidation in the presence of PEG-Cholesterol Oxidase enzyme and hydrogen peroxide (H2O2) is obtained. The H2O2 reacts with 4-aminoantipyrine under the catalysis of peroxidase enzyme to form a colored product. The color intensity formed as a result of this reaction and measured at specific wavelengths is directly proportional to the concentration of cholesterol in the sample.

REAGENT COMPONENTS

Reagent 1:

Dextran Sulfate	≤ 10 gr/dL
Magnesium Chloride Hexahydrate	≤ 5 gr/dL
Preservative	
Brij 35	≤ 10 gr/dL

Reagent 2:

Detergent	≤ 2 %
PEG-Cholesterol Esterase	≤ 5 KU/L
PEG- Cholesterol Oxidase	≤ 5 KU/L
4 amino-antiprine	≤ 1 gr/dL
Peroxidase	≤ 8000 U/L

REAGENT PREPARATION

Reagents are 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 30 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.[3]

SAMPLE REQUIREMENTS

Serum and plasma can be used and are collected according to the standard procedures. For plasma, sample collection tubes with Li heparin, K2 EDTA and K3 EDTA should be preferred.
It is recommended to use samples taken on an empty stomach.

HDL activity stability in serum and plasma:

Serum:
3 days at +20/+25ºC,
7 days at +2/+8ºC,
12 months at -20°C.

Plasma:
3 days at +20/+25ºC,
7 days at +2/+8ºC,
3 months at -20°C.

Unit Conversion:

mmol/L × 38.67 = mg/dL
mg/dL × 0.02586 = mmol/L

CALIBRATION AND QUALITY CONTROL

Calibration: The assay requires the use of an Archem HDL-LDL (Arcal Lipid) Calibrator.

HDL-LDL Calibrator (Arcal Lipid)-Lyophilized
Ref.No: A39048
Ref.No: A39049 (For Olympus AU series.)

Calibration stability is 30 days.Calibration stability depends on the application characteristics and cooling capacity of the autoanalyzer used.

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

Arcon N Level 1 Control- Lyophilized
Ref.No: A3910
Ref.No: A3912 (For Olympus AU series.)
Ref.No: A3913 (For BS series.)
Ref.No: A3914 (For Erba.)

Arcon P Level 2 Control- Lyophilized
Ref.No: A3920
Ref.No: A3922 (For Olympus AU series.)
Ref.No: A3923 (For BS series.)
Ref.No: A3924 (For Erba.)

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

Adult Men:	<35 mg/dL (0.90 mmol/L)	High risk
	>55 mg/dL (1.45mmol/L)	No CHD risk
Adult Women:	<45 mg/dL (1.15 mmol/L)	High risk
	>65 mg/dL (1.68mmol/L)	No CHD risk

For General Limits (Women and Men): 35-70 mg/dL

National Cholesterol Education Program (NCEP) guidelines:

< 40 mg/dL	: Low HDL (High CHD risk)
≥ 60 mg/dL	: High HDL (Low CHD risk)

CHD: Coroner Heart Disease

HDL cholesterol is affected by many factors such as smoking, sports, hormones, gender and age.

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 data have been verified by using CLSI EP28-A3c protocol.[4]

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).[5]

The determined analytic measuring interval for HDL is 3-200 mg/dL.

Detection Capability

Limit of Detection (LoD): 2.7 mg/dL

Limit of Quantitation (LoQ): 3.0 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.[6]

Linearity

This method shows measurement linearity up to 200 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 1:10 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.[7]

Precision

A precision study was performed according to CLSI EP05-A3:2014. 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.[8]

Repeatability (Within Run) and Repeatability (Day to Day) SD and CV% values of HDL have been given in the table 1 and 2 respectively.

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

Mean Concentration	SD*	%CV	n
60.1 mg/dL	0.61	1.01	80
131.1 mg/dL	0.79	0.60	80

***SD: Standard Deviation**

Note: This working system has been named "Within-Run Precision" in the previous CLSI - EP05-A2 manual.[9]

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

Mean Concentration	SD	%CV	n
60.4 mg/dL	1.23	2.05	80
131.1 mg/dL	2.86	2.18	80

Note: This working system has been named "Within-Run Precision" in the previous CLSI - EP05-A2 manual.[9]

Method Comparison

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

Passing-Bablock equation:
y= 1.015x+0.96 mg/dL
r=0.991

Interference

Interferant and Concentration	HDL Target (mg/dL)	N	Observed Recovery %
Hemoglobin 1260 mg/dL	25,8	5	91
Bilirubin 54 mg/dL	46,3	5	103
Lipemia 1062 mg/dL	53,6	5	111

Endogenous interferant and analyte concentrations that have been used in the HDL scanning tests has been determined according to "CLSI EP37-ED1:2018" and "CLSI EP07-ED3:2018" manuals.[10,11]

The total acceptable error rate, which is going to be used to detect whether the observed differential value obtained from HDL interference scanning test is appropriate, is determined as ±10%.[12]

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

* 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).[11]

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.[11]

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.
Contains sodium azide.

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 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.

REFERENCES

1. David L. Nelson and Michael M. Cox. Lipid Biosynthesis Chapter 21, In: Lehninger Priciples of Biochemistry 7th ed. Newyork: W. H. Freeman and Company 2017;2143-2266
2. Nader Rifai, G. Russell Warning and Alan T. Remaley. Lipids, Lipoproteins, Apolipoproteins and Other Cardiovascular Risk Factors Chapter 23 In: Tietz Fundamentals of Clinical Chemistry 6th ed. Philadelphia, PA: WB Saunders Company; 2008: 402-430
3. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline. CLSI Document EP25-A. Wayne, PA: CLSI; 2009
4. Clinical and Laboratory Standards Institute (CLSI). Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline – Third Edition. CLSI Document EP28-A3c. Wayne, PA: CLSI; 2010.
5. Clinical and Laboratory Standards Institute (CLSI). Establishing and Verifying an Extended Measuring Interval Through Specimen Dilution and Spiking – 1st Edition. CLSI Document EP34. Wayne, PA: CLSI; 2018.
6. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition. CLSI Document EP17-A2. Wayne, PA: CLSI; 2012.
7. Clinical and Laboratory Standards Institute (CLSI). Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach – 1st Edition. CLSI Document EP06-A. Wayne, PA: CLSI; 2003.
8. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition. CLSI Document EP05-A3. Wayne, PA: CLSI; 2014.
9. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline - Second Edition. CLSI Document EP05-A2. Wayne, PA: CLSI; 2004.

10. Clinical and Laboratory Standards Institute (CLSI). Supplemental Tables for Interference Testing in Clinical Chemistry – 1st Edition. CLSI Document EP37. Wayne, PA: CLSI; 2018.
11. Clinical and Laboratory Standards Institute (CLSI). Interference Testing in Clinical Chemistry – Third Edition. CLSI Document EP07. Wayne, PA: CLSI; 2018.
12. CLIA proficiency testing criteria for acceptable analytical performance, as printed in the Federal Register July 11, 2022;87(131:41194-242)

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SYMBOLS

IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
R1	Reagent 1
R2	Reagent 2
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