DIRECT HDL CHOLESTEROL

CE IVD

HDL

REF	Kit Size	
BC101-001	R1: 4 x 45 mL	R2: 4 x 15 mL

Intended Use:

For the quantitative determination of the concentration of High-Density Lipoprotein Cholesterol (HDL-C) in human serum or plasma.

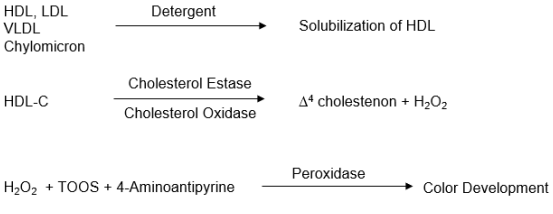
Test Summary

Cholesterol contained in the HDL (High Density Lipoprotein) fraction is called HDL-C. The results of the Framingham Study conducted in 1977 [1] indicated that a decreased level of HDL-C is a risk factor for atherosclerosis. The decreased HDL-C is associated with coronary heart disease, hyperlipidaemia, smoking, obesity, diabetes, and hepatic diseases and the concentration of HDL-C increases as a result of alcohol intake or moderate exercise. In addition, factors such as age, sex, and hereditary predisposition are known to affect the concentration.

The reference method for HDL-C quantification involves a combination of ultracentrifugation and chemical precipitation to separate HDL from other lipoproteins, after which cholesterol levels are measured using the Abell-Kendall method. In the past, common laboratory methods involved selective precipitation and elimination of LDL and VLDL, followed by enzymatic measurement of HDL-C in the remaining portion. These methods necessitated manual pre-processing and separation steps, making it challenging to fully automate the assay procedures. Consequently, the routine determination of HDL-C has been hindered by time-consuming procedures and suboptimal reproducibility.

Test principle

The method follows a two-reagent format and relies on a unique detergent's characteristics, as demonstrated. This approach is centered on expediting the reaction between cholesterol oxidase (CO) and non-HDL unesterified cholesterol, while selectively dissolving HDL using a specific detergent. In the initial reagent, non-HDL unesterified cholesterol undergoes an enzymatic reaction, and the resulting peroxide is consumed in a peroxidase reaction with TOOS, resulting in a colorless product. The second reagent includes a detergent that can specifically dissolve HDL, cholesterol esterase (CE), and a chromogenic coupler to produce color, facilitating the quantitative determination of HDL-C. This methodology can be referred to as the Accelerator Selective Detergent approach.



Reagents

Composition of Reagents

Constituents	**Reagent 1 (R1)** Good's buffer (pH 7.1) 4-Aminoantipyrine <0,01 % BSA <0,1 % Preservative <0,1 % **Reagent 2 (R2)** Good's buffer (Ph 6.7) TOOS < 0,01 % Preservative <0,1 % Cholesterol oxidase >5000 U Peroxidase >3000 U Cholesterol esterase >500 U

Warnings and Precautions for Use

Reagent 1:

Warning Contains: reaction mass of Sodium Azide
Hazard statements
H317 - May cause an allergic skin reaction
Precautionary statements
P261 - Avoid breathing dust/fume/gas/mist/vapors/spray.
P272 - Contaminated work clothing must not be allowed out of the workplace.
P280 - Wear protective gloves/protective clothing/eye protection/face protection.
P302+P352 - If on skin: Wash with plenty of water.
P321 - Specific treatment (see supplemental first aid instruction on this label).
P333+P313 - If skin irritation or rash occurs: Get medical advice/attention.

P363 - Wash contaminated clothing before reuse.
P501 - Dispose of contents/container to hazardous or special waste collection point, in accordance with local, regional, national and/or international regulation.

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Reagent 2:

Warning Contains: reaction mass of Sodium Azide
Hazard statements
H317 - May cause an allergic skin reaction
Precautionary statements
P261 - Avoid breathing dust/fume/gas/mist/vapors/spray.
P272 - Contaminated work clothing must not be allowed out of the workplace.
P280 - Wear protective gloves/protective clothing/eye protection/face protection.
P302+P352 - If on skin: Wash with plenty of water.
P321 - Specific treatment (see supplemental first aid instruction on this label).
P333+P313 - If skin irritation or rash occurs: Get medical advice/attention.
P363 - Wash contaminated clothing before reuse.
P501 - Dispose of contents/container to hazardous or special waste collection point, in accordance with local, regional, national and/or international regulation.
Do not pipette by mouth.
All specimens used in the test should be considered potentially infectious.
Universal precautions as they apply to your facility should be used for handling and disposal of materials during and after testing. Do not use the reagents after the expiration date printed on the reagent label.

Characteristics

1) This product is a reagent for measurement of HDL-C by the direct method.
2) The reagent does not contain magnesium (Mg) salts and can be used in relevant instruments without causing any damage.

Preparation of reagents and stability

Reagent 1: Ready to use.

Reagent 2: Ready to use.

Storage temperature: 2- 8°C

Shelf life: 15 months from the date of manufacturing

Disposal

Reagents must be disposed of in accordance with all Federal, Provincial, State, and local regulations.

Specimen

The required specimens for testing should be either serum or plasma obtained from the patient after a 12 to 14-hour fasting period.

For serum: Collect whole blood through venipuncture, allow it to clot, and then promptly centrifuge and separate the serum within 3 hours of collection.

For plasma: Specimens can be collected in EDTA, lithium, or sodium heparin. Centrifuge and separate the plasma as soon as possible within 3 hours of collection.
It's important to note that serum or plasma should not be left at temperatures between 18-28°C for more than 24 hours. If testing cannot be completed within this timeframe, store the serum or plasma at 2-8°C for up to 3 days. If longer storage is necessary, specimens can be preserved at temperatures below -20°C for up to a month, and samples can be frozen once. For more detailed instructions on specimen collection, handling, and storage, please refer to NCCLS Document H18-A.

Analytical Specificity

All interference studies were conducted according to a modified NCCLS guideline No. EP7 for interference testing in clinical chemistry.

Substances Tested	**Concentration with no significant (±10%) interference**
Hemoglobin	500 mg/dL
Triglycerides	3000 mg/dL
Bilirubin	20 mg/dL

Limitations

1. Anticoagulants containing citrate should not be used.
2. Protect the reagents from direct sunlight.
3. Store the reagents at 2-8°C. Do not freeze the reagents.
4. The NCEP recommends that dietary and/or drug treatment not be based on a single HDL cholesterol result.
5. Endogenous triglyceride levels gave acceptable performance up to 3000 mg/dL.
Samples with triglyceride level >3000 mg/dL should not be diluted.
6. Samples containing the following should not be used:
N-acetylcysteine (NAC).

General precautions

1. This product is for *in vitro* diagnostic use and must not be used for any other purpose.
2. When a clinical decision is made based on the test results obtained by using Cholesterol HDL, the clinician should also take into consideration the patient's clinical condition and findings on examination.
3. This product should be used as directed in this package insert. The reliability of the measurement values cannot be

guaranteed if Cholesterol HDL is used for purposes or tested by methods not stated in this document.
4. If the reagents contact with the eyes or mouth, rinse thoroughly with water as a first aid measure and consult a doctor if required.

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5. Carefully read the operating instructions for each type of automated analyzer.
6. After calibration, perform bilevel quality assessment.

Analytical Procedure

Wavelength	546/700 nm
Temperature	+37ºC
Measurement	Endpoint
Sample/Calibrator	10 µL
Reagent 1	900 µL
Reagent 2	300 µL
1st Incubation (R1+Sample)	5 minutes
2nd Incubation (After adding R2)	5 minutes
Calibration	Linear

Materials required but not provided

- HDL Calibrator
- HDL Control Level 1 & Level 2

*Any human cholesterol-based calibrator and control material can be used. After calibration, Quality Control values must be within the expected range. Quality control requirements should be established in accordance with local, state, and/or federal regulations or accreditation requirements.

**Performance studies were conducted with URIT 8210 test systems. Before measuring sample, please apply your own instrument operator's manual for analyzer specific procedures and for guidance in determining calibration frequency.

Calculation

$$\text{HDL-C [mg/dL]} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Cal.}} \times \text{Conc. Cal. [mg/dL]}$$

Conversion Factor

HDL-C [mg/dL] x 0.02586 = HDL-C [mmol/L]

Reference Intervals

HDL hypocholesterolemia[2)] is said to be present with levels of 40 mg/dl or lower.

National Cholesterol Education Program (NCEP) guidelines[2]

< 40 mg/dL Low HDL-Cholesterol (major risk factor for CHD)

>60 mg/dL High HDL-Cholesterol (negative risk factor for CHD)

Reportable Range

Linearity assessments were carried out using a human serum sample. The linearity simples were prepared via diluted human serum. JTC HDL kit has a linear response in the range of 1.0 mg/dL to 146 mg/dL, with a deviation from the linear trend of no more than 10%.

Patient samples with HDL cholesterol levels exceeding 146 mg/dL should be diluted with physiological saline before assaying. Multiply the result obtained from the manual dilution by the appropriate dilution factor.

Storage and Stability

Reagents are stable up to the date of expiry indicated on the kit, if stored at +2-8ºC and contamination is avoided. Do not freeze and protect from the light.

Precision

Within-run precision for the JTC HDL Cholesterol method was determined using three levels of frozen pooled human serum. Each run consisted of twenty replicate samples. Within-run precision studies produced the following results on the URIT 8210 Analyzer:

Serum Pool	Low	Mid	High
n	20	20	20
Mean (mg/dL)	35.9	50,8	62,4
Standard Deviation	0,88	0,77	0,49
CV%	2,44	1,51	0,78

Between-run precision for the JTC HDL Cholesterol method was determined using three levels of frozen pooled human serum. For 5 days, two different run was performed in a day as dublicated. Within-run precision studies produced the following results on the URIT 8210 Analyzer:

Serum Pool	Low	Mid	High
n	20	20	20
Mean (mg/dL)	35.6	51,5	61,8
Standard Deviation	0,88	0,76	0,79
CV%	2,48	1,48	1,27

Total error determination:

Total error serves as a comprehensive measure of an assay's overall analytical performance, encompassing both accuracy and precision. It can be calculated as the sum of % Bias and 1.96 times the Total Coefficient of Variation (%CV). The % Bias for the JTC HDL assay kit was determined through a linear regression formula, derived from a comparison with the Sekisui HDL assay kit. Below are the results of the total error analysis for the JTC HDL assay kit on the Urit automated analyzers at low, medium, and high HDL Cholesterol levels.

HDL cholesterol concentration	Bias%	Total CV%	Total Error

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35	2.%	2.48%	6.8 %
50	8.6%	1.48%	11.5%
62	5.1%	1.27%	7.6%

Accuracy

The accuracy of the JTC HDL Cholesterol assay was confirmed through a comparison with the Designated Comparison Method for HDL cholesterol, yielding the following results:

Method	JTC HDL assay kit	Sekisui HDL assay kit
n	50	50
Mean(mg/dl)	57	53.5
Range (mg/dl)	1-146 mg/dl	2.5-200 mg/dl
Correlation coefficient	y=0.99x+0.39 R^2 = 0.994	

Reproducibility

Coefficient of variation of ≤5% (within run)

Method comparison

A comparison of the human material with a commercial competitor kit product (x) gave the following result:

y = 0.99 x + 0.39; r = 0.999 Serum

y = 0.97 x + 0.28; r = 0.996 Plasma

Literature

1. Gordon, T, Castelli WP, Hjortland MC, et al. Am. J. Med. 1977;62:707-714
2. Guidelines for Diagnosis and Treatment of Atherosclerotic Cardiovascular Diseases 2002, Japan Atherosclerosis Society, P.5

	Manufacturer
IVD	In vitro diagnostic medical device
REF	Catalog Number
	Exclamation Mark

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